This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

1616
#31
3/17/03

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

OIPE
FEB 19 2003
PATENT & TRADEMARK OFFICE

RECEIVED
FEB 21 2003
TECH CENTER 1600/2900

In re Patent Application of

SCHNEIDER et al

Serial No. 09/115,963

Filed: July 15, 1998

Atty. Ref.: 1201-55

Group: 1616

Examiner: Hartley, M.

For: LONG-LASTING AQUEOUS DISPERSIONS OR SUSPENSIONS OF PRESSURE-RESISTANT GAS-FILLED MICROVESICLES AND METHODS FOR THE PREPARATION THEREOF

* * * * * * * * * * *

February 19, 2003

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

LETTER

Attached for the examiner's convenience are copies of the three literature articles identified as Exhibit 2 and discussed on pages 14 and 15 of the Amendment of January 31, 2003.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____
Arthur R. Crawford
Reg. No. 25,327

ARC:eaw
1100 North Glebe Road, 8th Floor
Arlington, VA 22201-4714
Telephone: (703) 816-4000
Facsimile: (703) 816-4100

Physical and biochemical stability of Optison®, an injectable ultrasound contrast agent

Sheila Podell[1], Cindy Burrascano, Marc Gaal, Brent Golec, Jack Maniquis and Paige Mehlhaff

Molecular Biosystems Inc., 10030 Barnes Canyon Road, San Diego, CA 92121–2789, U.S.A.

Optison® is an ultrasound contrast agent, consisting of gas-filled microspheres surrounded by a solid shell of heat-denatured human albumin. Size-distribution measurements of these microspheres are a critical stability indicating factor, because loss of encapsulated gas eliminates ultrasound contrast activity. Composition of the encapsulated gas is also critical, because air-filled microspheres do not persist nearly as long *in vivo* as microspheres filled with less soluble gases. Optison® stability has been tested during exposure to chemical substances expected to dissolve microsphere shells. In addition, size-distribution and gas-composition measurements were used to evaluate the effects of external gas composition, elevated temperature, mixing, needle shear and pressure on product stability. Optison® microsphere shells dissolve only when exposed to relatively extreme chemical conditions, such as low pH (< 4.0), detergents or chaotropic salts. The shells are highly gas-permeable, and microspheres lose encapsulated gas rapidly and irreversibly when exposed to gas-deficient liquids. Pressure, impact stress, and the application of ultrasound energy all cause liquids to become gas-deficient, and also cause irreversible gas loss. Pressure sensitivity differs dramatically between mixed and unmixed microspheres, further supporting the conclusion that gas diffusion is the major cause of Optison® instability. To preserve the efficacy of Optison® as an ultrasound contrast agent, it is necessary to devote special attention to minimizing opportunities for gas exchange, mixing and exposure to gas-deficient liquids, so that the size distribution and gas composition of the original product are maintained during handling.

Introduction

Optison® is an injectable ultrasound contrast agent, consisting of hollow albumin microspheres filled with octafluoropropane (OFP). The product is approved in the U.S.A. and Europe for use in left-ventricular opacification of the heart and improved delineation of left-ventricular endocardial borders, and is currently under investigation for additional indications in cardiology and radiology. Optison® is distributed in the U.S.A. and Europe by Mallinckrodt Medical, St. Louis, MO, U.S.A.

Ultrasound contrast is based on the principle of using sound waves to detect a difference in density between the contrast agent and surrounding tissue. Optimal density differences are obtained when the contrast agent is a gas, since tissues are primarily composed of liquid. The size of the microspheres is of great importance, because ultrasound signal (backscatter) increases with the 6th power of gas-bubble radius [1]. As a practical matter, however, ultrasound contrast agents intended for injection *in vivo* must be small enough to pass through blood capillaries without causing blockage (< 8 μm diameter). Consequently, the optimal size distribution for ultrasound contrast agents is a compromise between maximum backscatter and efficient capillary passage.

Creating and maintaining gas bubbles at a constant size is a technical challenge, because bubbles suspended in a liquid can shrink, grow or coalesce, and changes in gas-bubble size can occur very rapidly, within seconds to minutes [2,3]. Unencapsulated free gas bubbles were first used as ultrasound contrast agents as early as 1968 [4], but proved to be unsatisfactory because their size was uncontrolled, inconsistent and unstable. Once injected, they did not last long enough *in vivo* to allow an adequate ultrasound examination of target tissues, especially the left side of the heart.

Albunex®, the first ultrasound contrast agent approved for use in the U.S.A., addressed these problems by encapsulating air in a thin shell of heat-denatured protein [5]. These encapsulated microspheres were not only much more stable than naked bubbles *in vitro*, they also persisted longer *in vivo*. As a result, when injected intravenously, they were capable of passing from the right side of the heart through the lung circulation, to be visualized in the left side of the heart. In addition, the encapsulation process developed commercially by Molecular Biosystems (San Diego, CA, U.S.A.) allowed stringent control of the microsphere size distribution, so that the properties of the material injected

Abbreviations used: OFP, octafluoropropane; IEF, isoelectric focusing.

[1] To whom correspondence should be addressed.

© 1999 Portland Press Ltd

were known exactly and reproducible [6,7]. Despite these improvements over naked bubbles, the persistence of Albunex® *in vivo*, at 1–2 min, was considered too short to allow an optimal ultrasound examination, and additional improvements were pursued.

The second ultrasound contrast agent approved in the U.S.A. was Optison®, also manufactured by Molecular Biosystems. Optison® differs from Albunex® by substituting OFP for air as the core gas, while retaining heat-denatured human serum albumin as the encapsulating shell. Even though the albumin shell technology of Optison® is the same as Albunex®, Optison® persists much longer *in vivo*. This is because OFP is much less soluble than air in aqueous solutions. The lower solubility delays escape of encapsulated gas from the microsphere cores into the surrounding solution, prolonging gas retention in the microspheres [8].

Retention of gas in the microspheres, especially *in vivo*, has been a limiting factor in the development of new indications for ultrasound contrast agents, so the stability enhancement provided by OFP gas is a highly desirable property. In this light, it is not surprising that, in addition to Optison®, all other 'second-generation' ultrasound contrast agents currently in development also use insoluble fluorocarbon gases or vapours to generate acoustic backscatter (reviewed in [8,9]).

The activity of gaseous ultrasound contrast agents depends on their ability to deliver gas bubbles of appropriate size to a target site *in vivo*. Subtle changes in gas-bubble size distribution that could affect biodistribution or echogenicity are not routinely monitored by end users, and would not be detectable without access to special particle-sizing equipment. Given the well-known lability of gas bubbles, as well as the lack of visual markers for degradation, a sound understanding of the conditions required to maintain product stability during handling is necessary to ensure consistent product performance.

The purpose of this study has been to understand the mechanisms that contribute to Optison® stability and degradation during storage and handling. Both microsphere shells and core gases have been examined. The results of this study should have application to all studies performed with the product, including those aimed at determining dose response, route of administration, compatibility with new ultrasound equipment, comparisons with other ultrasound agents and utility for new clinical indications.

Materials and methods

Microsphere concentration and size distribution

Optison® and Albunex® microspheres were obtained from Molecular Biosystems. Product specifications for the two types of microsphere are shown in Table 1. Microsphere concentrations and size distributions were measured by electrical zone sensing with a Coulter Multisizer IIe (Beckman Coulter, Fullerton, CA, U.S.A.), according to the method described by Sontum and Christiansen [10], except that the 20-μl microsphere test samples were removed from intact vials directly through the septum closure, using a 19 gauge, 1 inch (2.5 cm) needle probe attached to a Hamilton Microlab 500 pipettor dilutor (Hamilton Co., Reno, NV, U.S.A.). Septum closures on the vials were preserved during microsphere sampling in order to avoid contamination of vial headspaces with air. Encapsulated gas volumes were calculated directly from size and concentration data obtained on the Coulter instrument. Gas-deficient diluents were obtained by vacuum degassing, and were mixed with air-saturated versions of the same diluent to achieve the desired degree of gas saturation. Final air-saturation levels were determined using an Orion model 862 oxygen meter.

Table 1 Comparison of Albunex® and Optison® microspheres

Specification	Albunex®	Optison®
Mean particle size	3.0–5.0 μm	2.0–4.5 μm
Microsphere concentration	(3.0–5.0) x 10^8/ml	(5.0–8.0) x 10^8/ml
Albumin content (w/v)	5% solution	1% solution
Core gas composition	Air	OFP
Size distribution	92.5% smaller than 10 μm	93% smaller than 10 μm

Gas composition

The gas composition of vial headspaces was assayed by removing a 10-μl sample of gas through the septum closure with a gas-tight glass syringe (part 1802, Hamilton Co.). The gas sample was injected on to a Hewlett Packard model 5890 gas chromatograph equipped with an Al_2O_3 column and a thermal conductivity detector, and was eluted from the column under conditions chosen to separate air from OFP. Relative molar percentages of air and OFP were determined by comparison of thermal conductivity detector response with known external reference standards.

To determine the gas composition of isolated microspheres, a well-mixed solution of microspheres was transferred into a headspace-free test apparatus, consisting of an evacuated 10-ml gas-tight glass syringe (part 81320, Hamilton Co.) fitted with a luer-connection septum adapter (part 31335, Hamilton Co.). Microsphere core gas was liberated by adding a small amount of concentrated solution of mixed alkyl trimethyl ammonium bromide (MTAB; M-7635, Sigma) through the septum adapter. The resulting free gas bubbles were consolidated by adding a small amount of ethanol to reduce surface tension. The final MTAB concentration was

© 1999 Portland Press Ltd

0.125%, and the final ethanol concentration was 5%. The newly formed headspace in the test syringe was analysed by gas chromatography, as described above.

Shell dissolution

Microsphere-shell dissolution studies were performed on intact vials with a septum closure. Each vial was first vented with a 20 gauge, 1 inch needle. With the vent needle still in place, 150 μl of 20 × concentrated test solution was added through a second needle attached to a Hamilton syringe. Needles of 20 gauge or larger were used routinely during microsphere manipulations to minimize back pressure during transfer. After the biochemical test solution was delivered, both needles were subsequently removed, and the sample mixed on a Fisher haematology mixer for 30 min. At the end of the 30-min mixing period, microsphere size distribution was measured using the Coulter Multisizer, as described above.

Electrophoretic characterization

Microsphere shells were isolated for electrophoretic characterization by first allowing intact microspheres to float, then removing the free albumin solution below the microsphere layer. The microspheres were then suspended in water and centrifuged at 1000 g for 10 min. The water layer was removed and the washing procedure was repeated twice more, for a total of three wash cycles. The absorbance of the wash solution at 280 nm was measured to insure that the washing steps were effective in removing free albumin. After the final wash, microsphere shells were gently disrupted using a hand-held glass/teflon homogenizer. The resulting solution was adjusted to a final protein concentration of 10 mg/ml, as determined by the Biuret protein assay [11].

SDS/PAGE was performed using Tris/glycine 8–16% gradient gels containing β-mercaptoethanol (Novex, San Diego, CA, U.S.A.). Gels were loaded with 2 μg of protein per lane, for both Coomassie Brilliant Blue and silver staining. Cyanogen bromide peptide mapping was performed on albumin and washed microspheres that were lyophilized to dryness. A 5.0-mg aliquot of each lyophilized sample was dissolved in 0.5 ml of 70% formic acid. Cyanogen bromide (4.77 mg) was added, and samples were placed in the dark for 24 h with occasional mixing. The reaction was stopped by the addition of 10 ml of water, then lyophilized to dryness. Cleaved samples were dissolved in SDS sample buffer at a final concentration of 0.5 μg/ml. Then, 20 μl of each sample (10 μg total) were loaded on to a 10–20% Tris/tricine SDS gradient gel (Novex).

Isoelectric focusing (IEF) was performed using precast gels (Novex). The gels were intentionally overloaded with 8 μg of sample to allow detection of faint bands. Fatty acid-free human albumin (Sigma) was included for comparison with the United States Pharmacopeia human albumin solution used for microsphere production, along with 20 μg of a pI standard mix (Novex). The gels were subjected to 100 V for 1 h, followed by 200 V for 1 h and finally 500 V for 30 min. The gels were fixed in 5-sulphosalicylic acid and the bands visualized with Coomassie Brilliant Blue.

Microscopic observations

Microscope observations were performed using a Nikon Optiphot-2 microscope, equipped with Nomarski optics. Microspheres were placed in a well slide under a coverslip, and allowed to float up to the coverslip surface. The slide was then perfused with water that was either air saturated or degassed, in order to observe the behaviour of the microsphere shells. Some microspheres were suspended in a 15-ml polypropylene centrifuge tube, and exposed to ultrasound energy from a Hewlett Packard model Sonos 100 ultrasound machine equipped with a 3.5 mHz transducer.

Shear, pressure and impact stress

For injection stress studies, well-mixed microspheres were pooled into a 140 ml polypropylene Monoject syringe (Sherwood-Davis & Geck, St. Louis, MO, U.S.A.), fitted with a 1.5 inch (3.75 cm) needle. The rate of microsphere injection was controlled using a Harvard Apparatus model 22 syringe pump (Harvard Apparatus, Holliston, MA, U.S.A.). The contents of the syringe were delivered via the needle through a septum adapter (part 31335, Hamilton Co.), directly into an empty 10 ml polypropylene syringe. As liquid was transferred to the empty syringe, the plunger was allowed to slide back freely, to accommodate the expanding liquid volume. This collection method was chosen because it avoided exposing the injected microspheres to either pressure or air contamination.

Pressure experiments were performed on microspheres packaged in glass vials with septum closures. Optison® microspheres were packaged in 3.0-ml vials, and Albunex® microspheres were packaged in 5.0-ml vials. Vials were vented with a 20 gauge, 1 inch needle, to insure a starting pressure of 1 atm (1 bar). A digital pressure gauge (Cole Parmer Instruments) with a 20 gauge, 1 inch needle connection was then inserted through the vial septum. Pressure was applied through a second needle (20 gauge, 1 inch) attached to a 20 ml polypropylene syringe filled with air. The plunger of the syringe was depressed manually, pushing air into the vial, until a reading of 20 psi (138 kPa) was attained, then held for the desired time. Pressure was released gradually, venting the vial to bring pressure back to 1 atm. After exposure to pressure, vials were mixed gently on a Fisher haematology mixer for 10 min, then assayed for

© 1999 Portland Press Ltd

microsphere-size distribution and concentration using the Coulter Multisizer.

The effect of impact stress on Optison® microspheres was measured by dropping product vials down a 1-m length of 2.5-cm-diameter pipe on to a 2.5-cm piece of medium density fiberboard (Medex). After being dropped, vials were mixed for 10 min on a Fisher haematology mixer, then measured for microsphere size distribution and concentration on the Coulter Multisizer. To measure the effect of multiple drops on unmixed vials, the vials were allowed to sit undisturbed for 2 h after each drop, to allow microspheres to re-segregate by flotation before the next drop.

Results

Microsphere-shell characterization

The stability of Optison® and Albunex® microsphere shells to dissolution was evaluated by subjecting whole microspheres to the chemical conditions shown in Table 2. Both air-filled Albunex® and OFP-filled Optison® microspheres were dissolved by low pH, detergents and chaotropic salts, but not by high pH or reducing agents. Cationic detergents dissolved microsphere shells at lower concentrations than non-ionic detergents or anionic detergents. There were some minor differences in the threshold of chemical concentration required for microsphere disruption within a 30-min time limit, which may be due to the higher concentration of free albumin in the Albunex® formulation (5%, w/v), compared with the Optison® formulation (1%, w/v). The overall profiles of Albunex® and Optison® dissolution were very similar, indicating that their albumin shells are held together by the same types of chemical forces.

The protein properties of albumin microsphere shells were investigated by several electrophoretic methods, as shown in Figure 1. Shells from both air-filled and OFP-filled microspheres appeared identical to untreated albumin by SDS/PAGE and cyanogen bromide peptide mapping, indicating that primary structure and peptide bonds of the albumin starting material were unaltered by shell formation. Human serum albumin is known to migrate in different IEF patterns, depending on the quantity of bound fatty acids [12]. The albumin raw material used to manufacture the microspheres displayed an IEF banding pattern typical of fatty acid-rich albumin, but the IEF pattern of the albumin in microsphere shells was more characteristic of fatty acid-deficient albumin. This result suggests that fatty acids bound to the original albumin raw material may not have been efficiently incorporated into microsphere shells, in agreement with previous work on Albunex® microspheres [13].

Effects of gas diffusion on microsphere stability

The physical properties of individual Optison® microspheres were assessed by microscopic observation. Microspheres diluted in air-saturated water or PBS appeared spherical, with a smooth surface, as shown in Figure 2(a). This appearance did not change when observed over a period of several hours. When exposed to gas-deficient liquids,

Table 2 Chemical stability of gas-filled albumin microspheres, after 30 min of continuous mixing at 25 °C

MTAB, mixed alkyl trimethyl ammonium bromide.

Chemical treatment	Final composition	Gas volume retained (%)	
		Optison®	Albunex®
Low pH	pH 2.0 (50 mM citrate)	51	4
	pH 3.0 (50 mM citrate)	90	6
	pH 4.0 (50 mM citrate)	100	33
High pH	pH 11.0 (50 mM dimethylamine)	100	100
	pH 12.0 (50 mM phosphate)	100	100
Cationic detergent	0.50% MTAB	0	2
	0.10% MTAB	2	3
	0.05% MTAB	14	63
Non-ionic detergent	0.50% Triton X-100	21	4
	0.10% Triton X-100	20	3
	0.05% Triton X-100	62	82
Anionic detergent	0.50% SDS	6	2
	0.10% SDS	71	93
	0.05% SDS	100	100
Reducing agent	500 mM β-Mercaptoethanol	99	89
	100 mM Dithiothreitol	100	100
Chaotropic salt	6.0 M Guanidinium chloride	4	3
	3.0 M Guanidinium chloride	94	52
Control	PBS, pH 7.2	100	100

© 1999 Portland Press Ltd

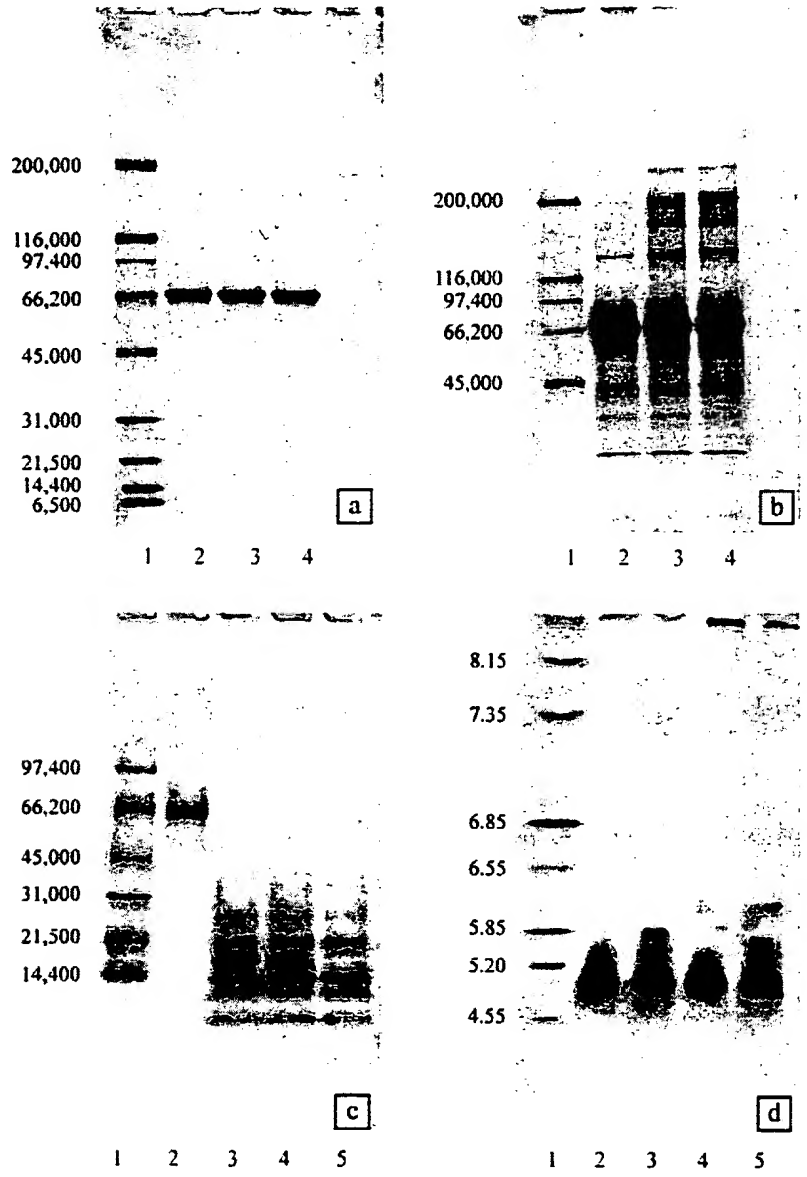

Figure 1 Electrophoretic characterization of microsphere-shell proteins

(a) Coomassie Brilliant Blue- and (b) silver-stained SDS/PAGE (8–16% gels). Lanes 1, molecular-mass standards (Da); lanes 2, United States Pharmacopeia human albumin used for production of microspheres (HA); lanes 3, air-filled microspheres; lanes 4, OFP-filled microspheres. (c) Coomassie Brilliant Blue-stained SDS/PAGE (10–20% gels). Lane 1, molecular-mass standards; lane 2, uncleaved HA; lane 3, CNBr-cleaved HA; lane 4, CNBr-cleaved air-filled microspheres; lane 5, CNBr-cleaved OFP-filled microspheres. (d) Coomassie Brilliant Blue-stained IEF gel. Lane 1, pI standard mix; lane 2, fatty acid-free human albumin (Sigma); lane 3, HA; lane 4, air-filled microspheres; lane 5, OFP-filled microspheres.

microsphere shells began to collapse within 1–5 min, as shown in Figures 2(b)–2(d). For each individual microsphere, shell collapse occurred in discrete, discontinuous stages, rather than in a uniform, gradual manner. Collapsed shell material was never observed to re-inflate, even when partially crinkled microspheres were perfused with fresh air-saturated solution. Not all microspheres in a given field collapsed simultaneously; larger microspheres appeared to collapse sooner than smaller ones. These results suggest that the shell may be somewhat inelastic, and require a certain threshold of stress before changing its shape. Microspheres exposed to pressure and ultrasound energy were identical in appearance to those exposed to degassed buffer. These results are consistent with the fact that pressure treatment and exposure to ultrasound energy both cause liquids to become gas deficient, and suggest that gas

© 1999 Portland Press Ltd

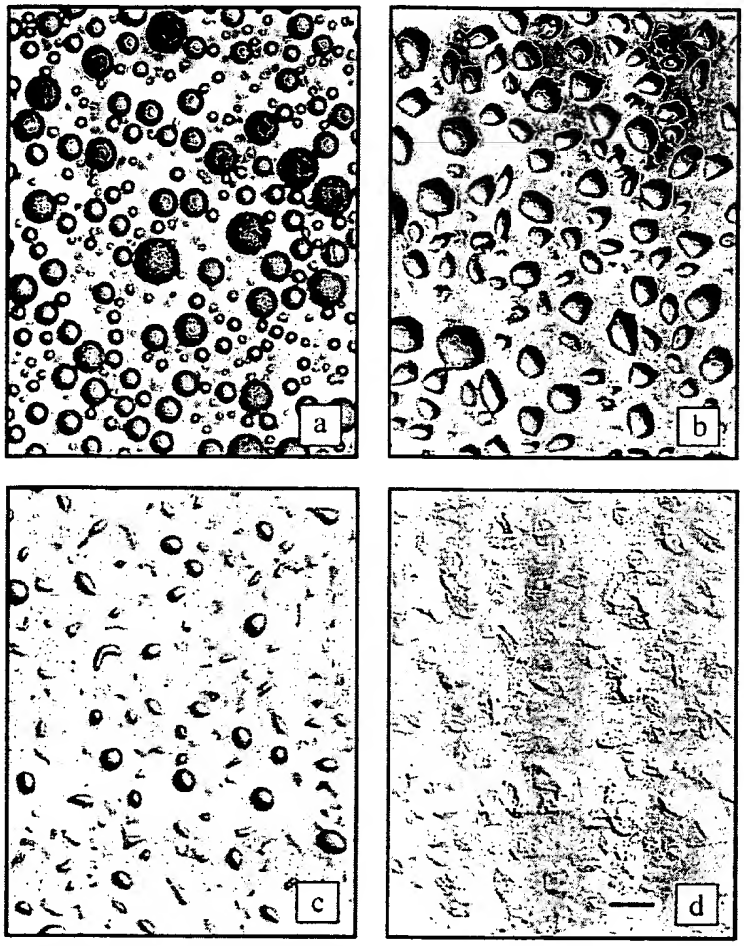

Figure 2 Photomicrographs of Optison® diluted in (a) air-saturated solution, (b) gas-deficient solution after 1.5 min, (c) gas-deficient solution after 4 min and (d) gas-deficient solution after 6 min

Images are from different fields, but scale is identical in all photos. Scale bar (**d**), 10 μm.

loss from microsphere cores may be the primary mechanism of microsphere destruction in the presence of these forces.

The effect of degassed liquids on Optison® microspheres was also investigated by measuring the size distribution of entire microsphere populations, using Coulter Multisizer measurements. The results, shown in Figure 3, confirm that microspheres rapidly lose their core gas when exposed to a gas-deficient environment. The timing of gas loss was more rapid when microspheres were diluted in the larger volume required for size-distribution measurements, compared with the relatively small dilution factor used for microscopic examination. In air-saturated diluent, microsphere-size distribution was relatively stable after the first minute, but in gas-deficient solutions microsphere size continued to decrease rapidly, with total elimination of encapsulated gas within a 5-min period.

The rapid loss of microsphere core gas observed in degassed solutions suggests that albumin microsphere shells are permeable to gas diffusion. This idea was tested directly by measuring the gas composition of two sets of microspheres, exposed to headspaces of differing compositions. The first set of microspheres, with an initial core gas composition of 83% OFP/17% air, was filled into vials under a headspace of pure air. The second set of microspheres, with an initial core gas composition of 52% OFP/48% air, was filled into vials under a headspace of 85% OFP/15% air. Comparison of headspace gas compositions before and after microsphere lysis indicate that microsphere cores that differ initially from the headspace reach equilibrium within about 24 h (Figure 4). When measured directly, the OFP content of microspheres stored under an air atmosphere decreased, and the OFP content of microspheres stored in a high-OFP atmosphere increased, with the same kinetics as changes in the headspace gas. These results confirm that both air and OFP can move freely, both into and out of microspheres. Under the carefully controlled conditions of this experiment,

© 1999 Portland Press Ltd

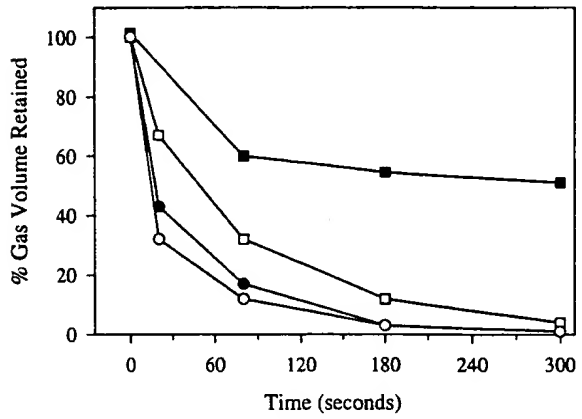

Figure 3 Effect of diluent gas saturation on microsphere stability

Air saturation: ■, 100%; □, 90%; ●, 60%; and ○, 22%.

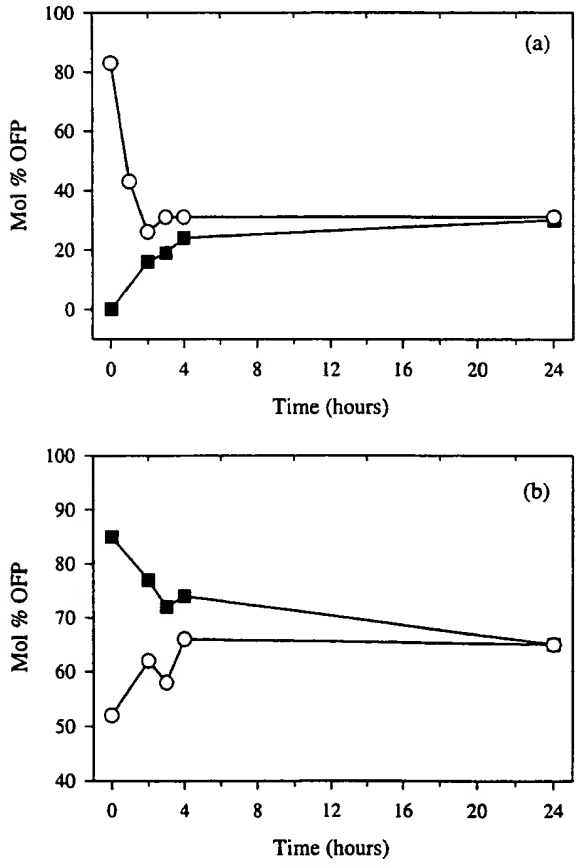

Figure 4 Gas composition of headspace and microspheres

■, Headspace composition; ○, microsphere composition. (a) Initial conditions of 0% OFP headspace/83% OFP microspheres. (b) Initial conditions of 85% OFP headspace/52% OFP microspheres.

with closed unmixed vials at room temperature, headspace and microsphere core gas compositions reached equilibrium after a period of about 24 h. In actual practice, the time

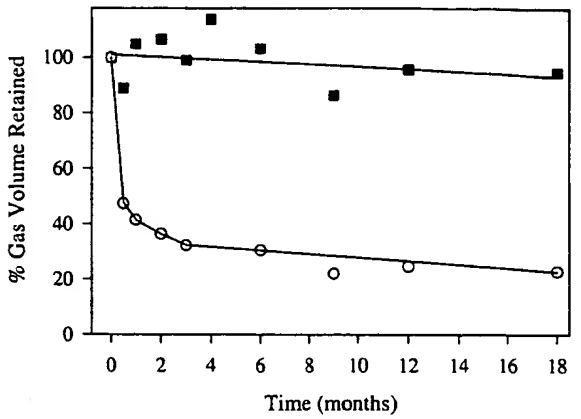

Figure 5 Effect of temperature on Optison® microsphere stability

■, Storage at 5 °C; ○, storage at 30 °C.

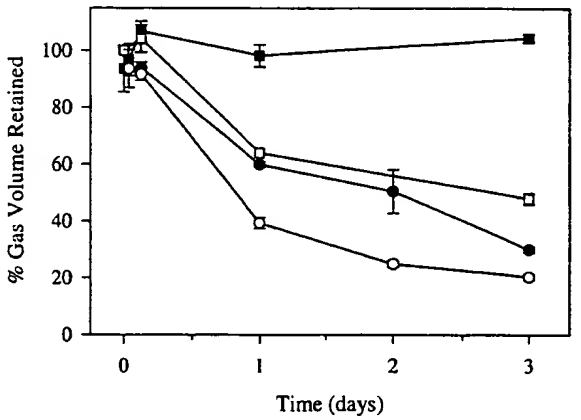

Figure 6 Effect of continuous mixing on Optison® microsphere stability

■, Unmixed, 5 °C; □, mixed, 5 °C; ●, mixed, 25 °C; ○, mixed, 45 °C. Error bars represent S.D. of triplcate measurements.

required to reach equilibrium between the two compartments would be expected to vary with temperature, mixing and magnitude of the differences in gas composition between microspheres and headspace.

Temperature and mixing

Optison® microspheres are stable, when stored under recommended conditions (5 °C, unmixed and unopened), for at least 18 months after their date of manufacture. They are considerably less stable when stored over the same period at 30 °C (Figure 5). Microspheres are stable on mixing for several hours, but begin to lose their gas after periods of a day or more, especially if mixing is combined with elevated temperatures (Figure 6). Freezing is not recommended, tested or approved for clinical use, but one cycle of freeze/thawing at −70 °C did not affect *in vitro*

© 1999 Portland Press Ltd

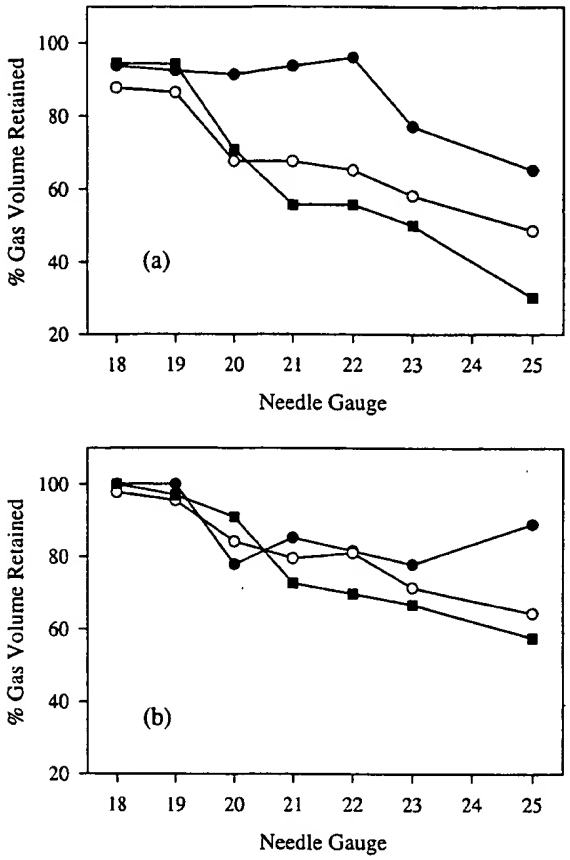

Figure 7 Effect of needle gauge on microsphere stability

Injection rate: ●, 0.5 ml/s; ○, 1.0 ml/s; ■, 1.5 ml/s. (a) Albunex® microspheres; (b) Optison® microspheres

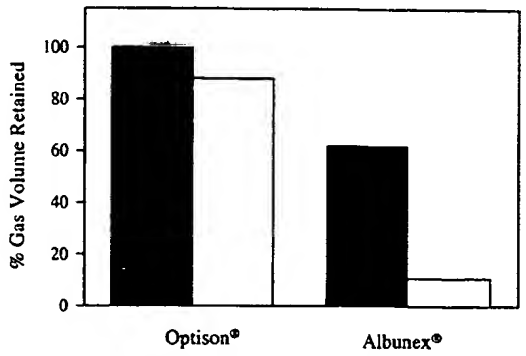

Figure 8 Effect of core gas composition on microsphere-pressure stability

Black bars, unmixed microspheres; white bars, mixed microspheres.

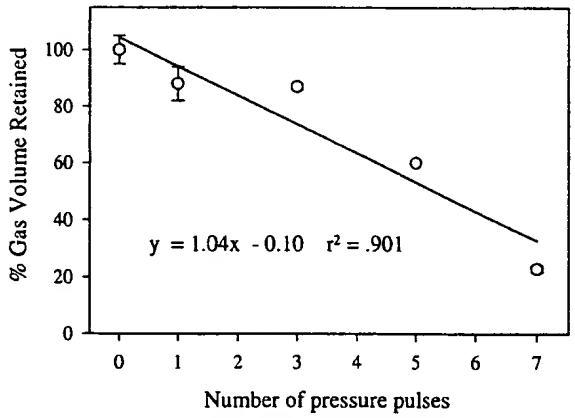

Figure 9 Effect of multiple pressure pulses of 20 psi on Optison® microsphere stability

measurements of Optison® size distribution, concentration or gas composition.

Shear, pressure and impact

During routine injections, shear forces, as well as pressure, are applied to microspheres as they are delivered. Recommended injection rates are no more than 1.0 ml/s, through a needle of 20 gauge or larger. The size distributions of well-mixed suspensions of Optison® and Albunex® were measured before and after injection through 1.5 inch needles at 0.5, 1.0 and 1.5 ml/s (Figure 7). Under equivalent conditions of shear stress, Optison® microspheres were found to be more stable than Albunex® microspheres. Optison® retained 86% of its original gas volume when injected at 1 ml/s through a 20 gauge needle, but Albunex retained only 68% of its original gas under the same conditions. Differences between Optison® and Albunex® were magnified by faster injection rates. Gas volume after injection declined for both Optison® and Albunex® as needle gauge increased, consistent with the fact that smaller needles increase both shear stress and back pressure.

The sensitivity of air-filled Albunex® microspheres to disruption by static pressure has been described in recent echocardiography literature [14–19], but the extent to which Optison® follows the same pattern has not been quantified. Figure 8 shows that the responses of both Albunex® and Optison® to pressure are highly dependent on whether the microspheres are mixed or unmixed at the time that pressure is applied. When exposed to pressure of 20 psi for 10 s, Optison® retained 100% of its original volume if unmixed, but only 88% if mixed. Under the same conditions, Albunex® retained 62% of its original volume if unmixed, but only 11% of its original volume if pressurized while mixed. These results are consistent with previous literature claims that Albunex® microspheres at higher concentrations are less sensitive to pressure [15]. Extending the duration of the pressure pulse from 10 to 30 s had no effect on the results for either type of microsphere (results not shown). When 10 s pressure pulses were applied

© 1999 Portland Press Ltd

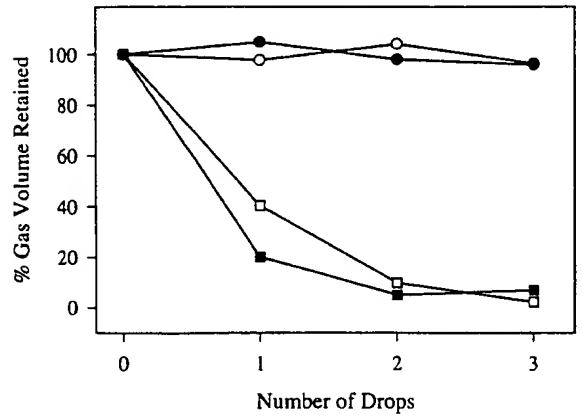

Figure 10 Effect of vial mixing on microsphere response to impact stress

○, Unmixed Optison®; ●, unmixed Albunex®; □, mixed Optison®; ■, mixed Albunex®.

repeatedly to a single vial of mixed microspheres, gas loss after each pulse was additive, in a roughly linear pattern (Figure 9).

If a vial of ultrasound contrast agent is dropped during routine handling, microspheres experience a brief, but intense, pressure pulse on impact. Like the responses to static pressure shown in Figures 8 and 9, microsphere response to the pressure wave generated by vial dropping is highly dependent on whether the vial is mixed or unmixed at the time of impact (Figure 10). After a single drop from a height of 1 m, the encapsulated gas volume of unmixed Optison® was unchanged, but mixed vials lost 60% of their gas. Albunex® microspheres were also resistant to destruction when unmixed, but even more sensitive than Optison® to gas loss when mixed. Multiple drops had an additive effect on mixed vials, but no effect on unmixed vials, as long as microspheres were allowed to return to fully layered form before the next drop occurred.

Discussion

The solid albumin shell surrounding both Albunex® and Optison® is important for providing stringent control of microsphere size at the time of manufacture, maintaining a constant size distribution during long-term storage, and allowing precise control of dosage administered at the time of injection. If the shell is dissolved chemically, the encapsulated gases dissipate rapidly, and migrate out of the liquid into the headspace. One possible mechanism by which a solid shell could stabilize encapsulated gas is by preventing direct contact between the core gas and the surrounding liquid, thereby eliminating Laplace pressure due to surface tension. Encapsulation with a solid shell also prevents coalescence and disproportionation, two other forces responsible for size changes and reduced longevity of non-encapsulated bubbles [20].

Relatively harsh conditions and long periods of time are required to dissolve albumin microsphere shells, suggesting that chemical dissolution is probably not the rate-limiting factor determining longevity of the ultrasound signal produced *in vivo*, which lasts only a few minutes. Gas diffusion appears to be much more important than shell biochemistry in determining the stability of gas-filled albumin microspheres. Direct measurements have demonstrated that gases from the Optison® microsphere core are in dynamic equilibrium with headspace gas, and that the albumin shell does not restrict gas diffusion between the microsphere cores and the surrounding environment. One practical application of this result is that headspace gas measurements can be used to determine microsphere gas composition in a closed vial, as long as sufficient time has elapsed for gas diffusion to reach a state of equilibrium. A second consequence is that for long-term storage, Optison® microspheres need to be packaged under a headspace containing the same gas composition that is desired for ultimate use *in vivo*. To prevent Optison® from acquiring the undesirable reduced stability of air-filled microspheres (e.g. Albunex®), its OFP content must be maintained by avoidance of gas exchange with air in the surrounding environment.

The biochemical properties of OFP-filled Optison® microsphere shells are virtually identical to those of air-filled Albunex® shells, yet Albunex® is far less effective as an ultrasound contrast agent than Optison®, due to lack of persistence *in vivo* [21,22]. The data presented here demonstrate that Albunex® microspheres are also more susceptible than Optison® to destruction by pressure *in vitro*. Gas solubility is known to increase linearly with the magnitude of pressure applied (Henry's law; see [23]). As pressure increases the capacity of a solution to accept dissolved gas, this capacity is saturated by gas drawn preferentially from microsphere cores, rather than the surrounding headspace. This preferential depletion occurs because the microspheres have a much higher surface/volume ratio, with correspondingly more opportunity for contact with the surrounding liquid. Differences in pressure sensitivity between Optison® and Albunex® lend support to the idea that gas solubility is the primary force responsible for microsphere destruction under pressure, since air is much more soluble than OFP in aqueous solutions. The role of gas solubility as a driving force for pressure sensitivity is further reinforced by data showing that gas loss is dependent on local microsphere concentration (e.g. whether mixed or unmixed), that destruction from multiple pressure pulses of the same magnitude is additive, and that microsphere destruction is independent of the duration for which pressure is applied.

© 1999 Portland Press Ltd

Air and OFP have been shown to move both in and out of microsphere cores. Theory predicts that each gas should move independently, at a rate determined by aqueous solubility, diffusibility and concentration gradient [2,24,25]. In air-saturated, OFP-poor solutions, air should enter Optison® microspheres faster than OFP leaves, leading to increased internal pressure in the microsphere core [26]. For large, non-encapsulated gas bubbles of pure OFP, a volume increase of up to 500% has been demonstrated in air-saturated solutions, where air can enter the bubble phase faster than OFP can leave [27]. In contrast, no such swelling was observed for Optison® microspheres when exposed to air-saturated solutions, either microscopically or by Coulter counting. These results suggest that the solid shell surrounding Optison® is capable of resisting the buildup of internal pressure generated by differences in gas-diffusion rates, and preventing microsphere expansion. Other ultrasound contrast agents, containing insoluble gases but lacking solid shells, would not be expected to show comparable resistance to expansion.

When Optison® is exposed to gas-deficient solutions, loss of gas from microsphere cores appears to be irreversible. The irreversibility of gas loss is an asset in preventing capillary blockage *in vivo*, but may cause unsuspected artifacts in studies where microspheres are removed from their original vials and manipulated in a research setting. Dilution of gas-filled microspheres has frequently been described in the echocardiography literature, without reference to gas saturation of the diluent solution, or size distribution of the microspheres after dilution. Gas undersaturation is common in laboratory solutions and commercial saline preparations, especially if the solutions have been autoclaved, or exposed to other temperature changes. The ease with which gases traverse the microsphere shell means that if microspheres are diluted into a non-gas-saturated solution, encapsulated gas could fall below the threshold level required for detection of ultrasonic backscatter within a matter of seconds to minutes.

Understanding the response of Optison® microspheres to gas-deficient solutions is useful for explaining the limited persistence of ultrasound contrast *in vivo*. Ultrasound contrast agents are typically injected intravenously, into blood that is gas-deficient with respect to all gases except nitrogen. In addition, gas deficiency of the environment *in vivo* is magnified by exposure to ultrasonic energy, as well as pulsatile pressure from the pumping action of the heart. In combination, these gas-depleting factors effectively promote gas diffusion out of the microspheres, allowing core gas to dissolve into the surrounding solution. Dispersed gases dissolved in solution no longer provide an ultrasound contrast effect, and are rapidly eliminated through the lungs. The sensitivity of Optison® microspheres to gas diffusion is a feature that all gas- or vapour-based ultrasound contrast agents share in common, since their echogenicity universally depends on delivering gas bubbles of a minimum size to target sites *in vivo*.

References

1 de Jong, N. and Hoff, L. (1993) Ultrasonics **31**, 175–181
2 Epstein, P. S. and Plesset, M. S. (1950) J. Chem. Phys. **18**, 1505–1509
3 Ward, C. A., Rizk, M. N. and Tucker, A. S. (1982) J. Chem. Phys **76**, 5606–5614
4 Gramiak, R. and Shah, P. M. (1968) Invest. Radiol. **3**, 356–366
5 Myrset, A. H., Nicolaysen, H., Toft, K., Christiansen, C. and Skotland, T. (1996) Biotechnol. Appl. Biochem. **24**, 145–153
6 Barnhart, J., Levene, H., Villapando, E., Maniquis, J., Fernandez, J., Rice, S., Jablonski, E., Gjoen, T. and Tolleshaug, H. (1990) Invest. Radiol. **25**, S162–S164
7 Christiansen, C., Kryvi, H., Sontum, P. C. and Skotland, T. (1994) Biotechnol. Appl. Biochem. **19**, 307–320
8 Jablonski, E. G., Dittrich, H. C., Bartlett, J. M. and Podell, S. B. (1998) Rev. Prog. Quant. Nondistruct. Eval. **17**, 15–22
9 Cotter, B., Mahmud, E., Kwan, O. I. and DeMaria, A. N. (1997) in Ultrasound Contrast Agents (Goldberg, B. G., ed.), pp. 31–42, Martin Dunitz, London
10 Sontum, P. C. and Christiansen, C. (1994) J. Pharmaceut. Biomed. Anal. **12**, 1233–1241
11 Bailey, J. L. (1962) in Techniques in Protein Chemistry, pp. 340–341, Elsevier, New York
12 Basu, S. P., Rao, S. N. and Hartsuck, J. A. (1978) Biochim. Biophys. Acta **533**, 66–73
13 Hellebust, H., Christiansen, C. and Skotland, T. (1993) Biotechnol. Appl. Biochem. **18**, 227–237
14 Shandas, R., Sahn, D. J., Bales, G., Elkadi, T., Yav, K. K. and Charib, M. (1990) Circulation **82**, III–95
15 Wiencek, J. G., Walker, R., Gretler, D., Montano, A., Jones, K., Harper, P. V., Aronson, S. and Feinstein, S. B. (1992) J. Am. Coll. Cardiol. **19**, 175A
16 Vuille, C., Nidorf, M., Morrissey, R. L., Newell, J. B., Weyman, A. E. and Picard, M. H. (1994) J. Am. Soc. Echocardiogr. **7**, 345–354
17 Mor-Avi, V., Shroff, S. G., Robinson, K. A., Ng, A. F., Cholley, B. P., Marcus, R. H. and Lang, R. M. (1994) J. Am. Coll. Cardiol. **24**, 1779–1785
18 Gottlieb, S., Ernst, M. and Meltzer, R. S. (1995) J. Ultrasound Med. **14**, 109–116
19 Padial, L. R., Chen, M. H., Vuille, C., Guerrero, J. L., Weyman, M. D. and Picard, M. H. (1995) J. Am. Soc. Echocardiogr. **8**, 285–292
20 Ronteltap, A. D., Damste, B. R., De Gee, M. and Prins, A. (1990) Colloids Surfaces **47**, 269–283
21 Killam, A. and Dittrich, H. C. (1997) in Ultrasound Contrast Agents (Goldberg, B. G., ed.), pp. 43–55, Martin Dunitz, London

© 1999 Portland Press Ltd

22 Cohen J. L., Cherif J., Segar D. S., Gillam L. D., Gottdiener, J. S., Hausnerova, E. and Bruns, D. E. (1998) J. Am. Coll. Cardiol. **32**, 746–52
23 Fogg, P. G. T. and Gerrard, W. (1991) Solubility of Gases in Liquids: a Critical Evaluation of Gas/Liquid Systems in Theory and Practice, John Wiley and Sons, New York
24 Himmelblau, D. M. (1964) Chem. Rev. **64**, 527–550
25 Ward, C. A. and Tucker, A. S. (1975) J. Appl. Phys. **46**, 233–238
26 Van Liew, H. D. and Burkhard, M. E. (1995) Invest. Radiol. **30**, 315–321
27 Crittenden, J. J., de Juan, E. and Tiedeman, J. (1985) Arch. Opthalmol. **103**, 831–834

Received 9 April 1999; accepted 13 July 1999

© 1999 Portland Press Ltd

Cardiac Imaging Using Optison

Leslie N. Clark, BS, and Howard C. Dittrich, MD

Optis n (human albumin micr spheres; Mallinckrodt Inc., San Diego, CA) is an injectable suspension contrast agent indicated for use in left-ventricular chamber opacification and endocardial-border delineation. Substantial proportions of patients undergoing echocardiography have inadequate endocardial delineation and, therefore, wall motion (including stress echocardiography) without contrast. The extent of use of Optison for its current indications is likely to vary, and its use will depend upon the patient population and image quality obtained from noncontrast examinations. Early reports exist of its use in as many as 60% of patients undergoing studies in a given echocardiography laboratory. The rate of acceptance for endocardial delineation in stress echocardiography appears to be particularly high, because of the higher proportion of technically challenging studies whether with fundamental r sec nd harmonic imaging. The ability to aid in differentiati n of potential artifacts from pathology in the cavity has also been reported. Clinical studies have been conducted or are currently underway to evaluate Optison in the assessment of acute and chronic ischemic coronary artery disease. Studies in patients with unexplained acute chest pain and during exercise and pharmacologic stress have evaluated the ability of Optison to detect perfusion abnormalities as well as wall-motion abnormalities. Th rapid evolution of ultrasound imaging modalities such as harmonic Doppler and broad-band imaging will further enhance Optison's ability to characterize ischemic heart disease patients. ©2000 by Excerpta Medica, Inc.

Am J Cardiol 2000;86(suppl):14G–18G

The second-generation ultrasound contrast agent, Optison (human albumin microspheres; Mallinckrodt Inc., San Diego, CA), received US Food and Drug Administration (FDA) and European Commission (EC) approval on December 31, 1997 and May 18, 1998, respectively. Optison is currently indicated for use in patients with suboptimal echocardiograms to opacify the left ventricle and to improve the delineation of the left-ventricular endocardial borders in patients with suboptimal noncontrast studies. It is, of course, necessary to visualize the endocardium adequately to evaluate regional and global myocardial thickening and wall motion, critical markers of ischemic coronary artery disease.

CHARACTERISTICS

Optison, injectable suspension, is a suspension of microspheres composed of 1% human albumin sonicated in the presence of the inert gas octaflouropropane. Each milliliter of Optison contains $5.0–8.0 \times 10^8$ human albumin microspheres with a mean diameter of 2.0–4.5 μm, of which 93% are <10 μm in diameter. Unlike other contrast agents under clinical development, Optison is fully manufactured before being filled into 3-mL single-use vials. No preparation of the product is required other than simply resuspending the microspheres into solution by gentle mixing. The product is then ready to be drawn up (with venting) into a syringe for single use.

The Optison microspheres, passing through the circulation, create a gas-liquid interface and resonate in response to ultrasound exposure, both of which result in a very potent echogenic effect within the intravascular space. To pass unimpeded through the circulation, the microspheres must be small enough (<7 μm), but large enough to maximize echoreflectivity because the reflectance is proportional to the radius of the microsphere to the sixth power.

IMAGING USING OPTISON

Endocardial-border delineation and left-ventricl opacification: Optison, the second-generation contrast agent, is superior to that of its predecessor Albunex and consistently and reproducibly improves visualization of left-ventricular endocardium for prolonged periods of time.[1–3]

The efficacy of Optison to reproducibly opacify the left ventricle and to improve the delineation of the left-ventricle endocardial borders was clearly demonstrated during pivotal clinical trials. The study included a total of 203 patients, all of whom had suboptimal images at baseline defined as at least 2 of 6 apical endocardial segments poorly visualized. Included in the population were 74 patients (37%) with clinically significant cardiomyopathy (ejection fraction 0.20–0.40) and/or chronic lung disease, a subgroup in whom imaging may be compromised or in

TABLE I Adverse Events Considered to Be Optison Related

	All Patients (n = 198)	Impaired Function Group (n = 73)
Transient altered taste	4 (2%)	1 (1.4%)
Flushing/warmth	4 (2%)	0
Nausea	0	0
Headache	1 (0.5%)	0
Injection site pain	1 (0.5%)	0
Dry mouth	0	0
Ecchymosis at IV site	0	0

IV = intravenous.

From Molecular Biosystems, Inc., San Diego, California, USA.

Address for reprints: Howard C. Dittrich, MD, Molecular Biosystems, 10030 Barnes Canyon Road, San Diego, California 92121.

 ©2000 by Excerpta Medica, Inc. All rights reserved.

0002-9149/00/$ – see front matter
PII S0002-9149(00)00984-X

whom the potential risk of adverse events might be greater. Finally, a subgroup of 85 patients was deemed by the core laboratory to have entirely nondiagnostic noncontrast images (≥4 of 6 endocardial segments poorly visualized). After Optison (3 mL), 63 (74%) of these patients converted to a diagnostic study (≥5 of 6 segments adequately visualized), demonstrating that Optison can yield a diagnostic study where noncontrast imaging would require the use of additional studies to resolve questions of cardiac function. A 0.5-mL dose of Optison improved ≥1 segment's visualization in 90% of all patients and in 87% of the impaired cardiac/pulmonary function group demonstrating equal efficacy in the latter, more difficult-to-image population. Importantly, adverse events considered to be related to the agent were low and mild regardless of whether they were evaluated in the group as a whole or in the impaired function subgroup (Table I).[1] It is key to remember that the imaging in this trial was performed with fundamental mode and real-time imaging, both of which limit contrast effect compared with the current expanding use of second harmonic, intermittent imaging. Recent studies have demonstrated the superiority of the newer imaging with Optison over the older technology and second harmonics without contrast.[4]

In recent studies, contrast echocardiography has been shown to improve significantly the evaluation of wall motion in intensive care unit patients. In a study of 29 patients, Optison was used in the intensive care unit where patients often have suboptimal echoes. Wall motion was evaluated by 2 blinded readers on fundamental frequency and compared with imaging on second harmonic and again after injection of Optison. Uninterpretable segments for each imaging modality were 35% for fundamental frequency, 30% for second harmonic, and only 7.5% after Optison. The conclusion drawn from the investigators was that contrast echocardiography significantly improves the evaluation of wall motion over either first or second harmonics without Optison (Figure 1).[5]

A secondary endpoint of the pivotal clinical trials of Optison was that of Doppler enhancement. At the end of each injection of Optison, when 2-dimensional imaging was completed, the left and right pulmonary veins were interrogated in 191 patients. Optison converted 79.3% and 48.0% of unreadable pulmonary Doppler signals to a diagnostic level in the left and right pulmonary veins, respectively (Figure 2). In addition, an improvement of ≥1 grades in image quality was noted at the 0.5-mL dose of 54.4% and 65.8% in the right and left pulmonary veins, respectively. These results demonstrate the potential utility for Optison in Doppler not only of the pulmonary vein, but also other valves such as the aortic valve in valvular stenosis.[6] Some laboratories report the ability to obtain maximal aortic-valve Doppler velocities more easily and in less time after Optison instead of prolonged multisite interrogation with blind Doppler probes.

Exercis str ss echocardiography: In recent studies, Optison has been shown to enhance images captured during exercise stress echocardiography.[7,8] In 1 study,

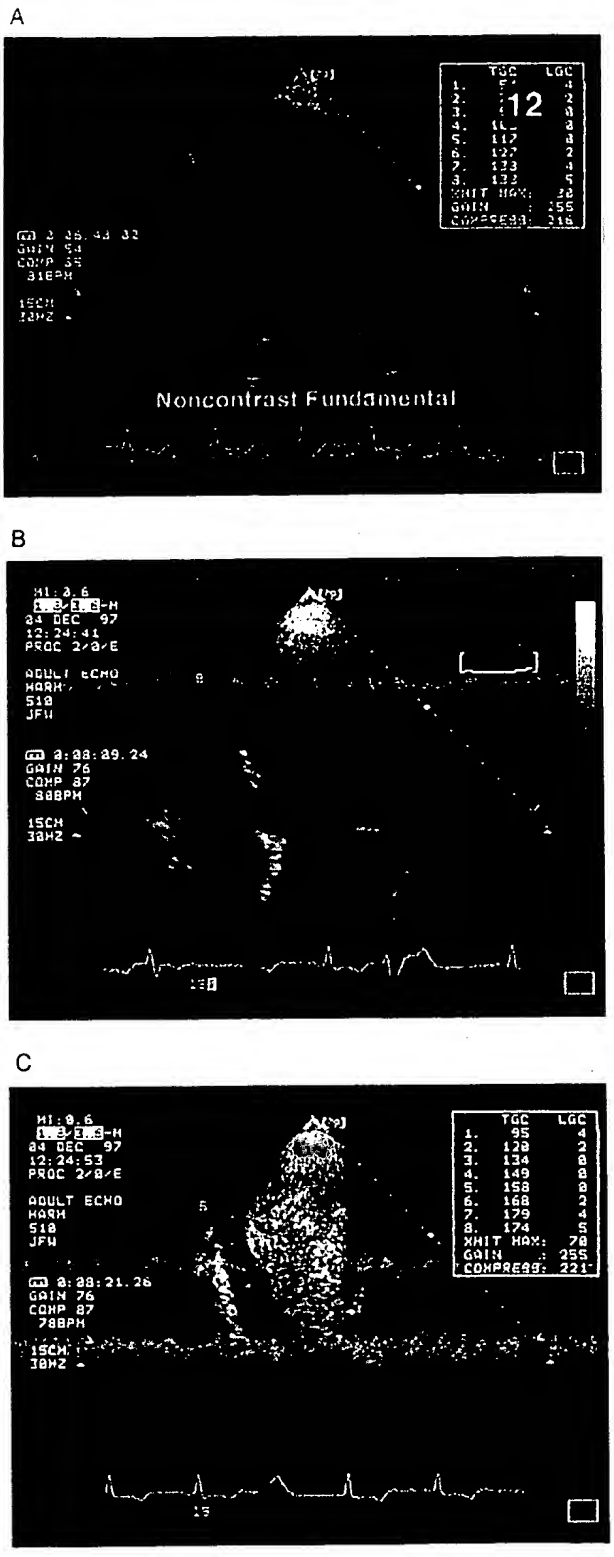

FIGURE 1. Patient imaged with noncontrast fundamental (*A*) and noncontrast second harmonics (*B*) was thought to have a dilated, thin-walled (0.9 cm) ischemic cardiomyopathy with an estimated ejection fraction of 0.15–0.25. After Optison (human albumin microspheres; Mallinckrodt, Inc., San Diego, CA) (*C*), the image demonstrates a much thicker myocardium with a smaller chamber and an estimated ejection fraction of 0.35–0.40. The Optison study changed the presumptive diagnosis to hypertensive cardiomyopathy.

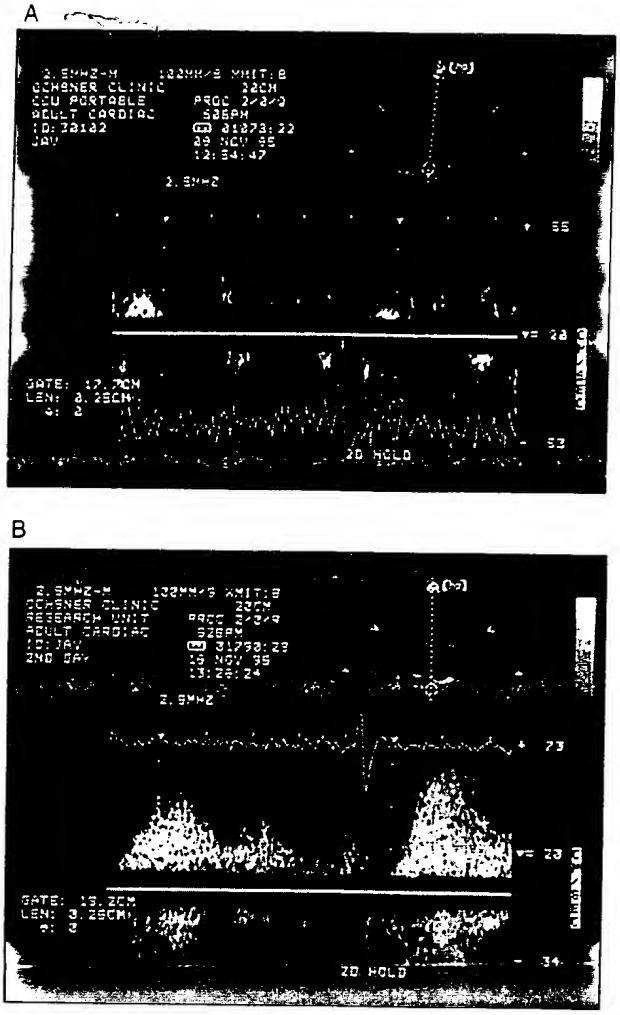

FIGURE 2. Right pulmonary vein pulsed wave Doppler before (*A*) and after (*B*) Optison.

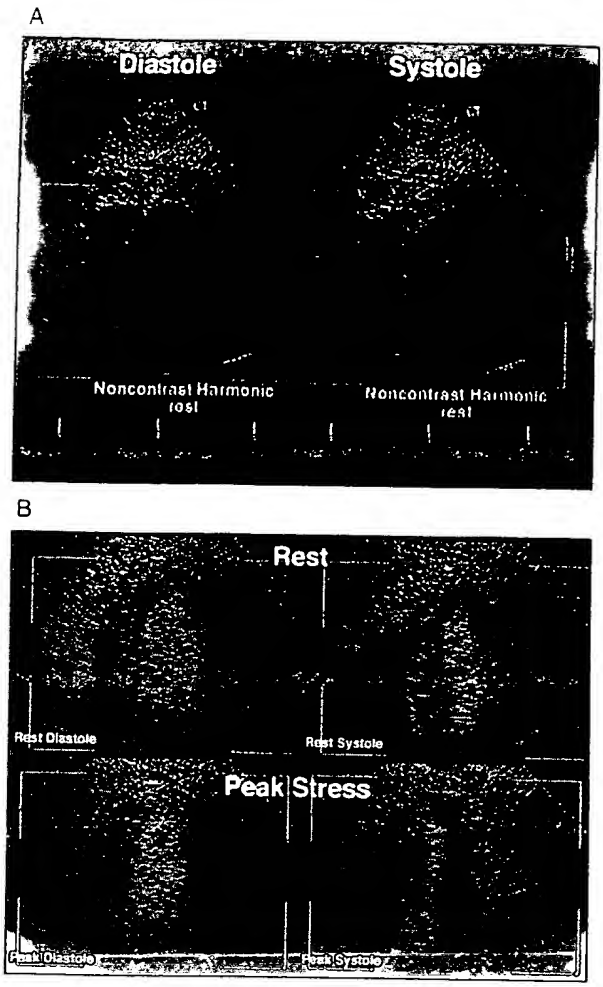

FIGURE 3. Second harmonic images from a patient undergoing stress echocardiography. The resting noncontrast images (*A*) are completely uninterpretable. After Optison (*B*), the rest and stress images show complete left-ventricular opacification and endocardial delineation, demonstrating normal wall motion.

58 patients unselected for image quality were stressed via bicycle or treadmill exercise and imaged in either first or second harmonic mode. A blind reader scored these images using a standard 18-segment echo model. Of the 2,064 segments scored, only 63.1% were interpretable without contrast. However, with Optison, 94.3% of the images were readable.[8]

Data analyzed from current phase II clinical studies also suggest that exercise stress echocardiography can be greatly enhanced with the use of Optison.[9] This study compared the Optison-enhanced exercise echocardiography with nuclear stress imaging in patients with nondiagnostic, noncontrast studies as determined by a blind reader. Specifically, the core lab identified 24 of 81 patients who, by the blinded reader, had such poor quality resting noncontrast studies that given the degradation of image quality with exercise, it would have been a waste of time and resources to proceed with exercise. In these 24 patients, the blinded reader would have referred the patient instead to nuclear single-proton emission computed tomography (SPECT) imaging. The Optison-enhanced rest and exercise studies were subsequently read and compared with nuclear SPECT exercise studies, either performed simultaneously or under similar work load. Assessment was made for fixed and reversible disease as well as extent of disease. In the case of Optison studies, a composite of wall motion and perfusion was used by blinded read to determine the presence or absence of disease. By myocardial segments, the endpoints of summed resting, summed stress, and difference from rest to stress, Optison agreed closely with SPECT with scores of 1.0, 3.0, and 1.7 versus SPECT scores of 1.9, 3.1, and 1.4. Assuming SPECT as a gold standard, Optison demonstrated an overall sensitivity and specificity for ischemia of 86% and 100%, respectively, in the 24 patients with unreadable, noncontrast rest images. These results clearly demonstrate that Optison exercise stress imaging can be applied accurately to a much broader population than is currently acknowledged (Figure 3).

Dobutamine stress echocardiography: Enhancement of image quality during dobutamine stress echocardiography with the use of Optison has also been demonstrated in several recent studies.[10-12] The addition

of Optison increases diagnostic ability and accuracy in the detection of coronary artery disease.[13]

The effect of Optison on sensitivity and specificity of dobutamine stress echocardiography was reported by Dolan et al.[13] A total of 204 patients with suboptimal second harmonic echoes were studied. Optison was administered to 92 patients and compared with 112 patients without contrast. No significant differences between patient group demographics were found. The results showed endocardial border delineation improvement from 52% ± 8% to 87% ± 7% after Optison. Sensitivity and specificity for detection of ≥70% stenosis by coronary arteriography were also improved with the administration of Optison (65% vs 89% and 72% vs 91%, respectively; $p < 0.01$). [14]

Myocardial perfusion: Optison has the potential to allow noninvasive assessment of myocardial perfusion, although it is not currently approved for this indication by regulatory authorities.[15–17] Myocardial perfusion assessment in patients with suspected coronary artery disease can be achieved by intravenous myocardial contrast echocardiography (MCE).[18–22]

A study involving 30 patients with known or suspected coronary artery disease was performed to determine whether MCE can detect coronary artery disease. These patients underwent both MCE and SPECT at baseline and after dipyridamole. Myocardial segment sections were scored for myocardial perfusion using background-subtracted, color-coded images from an early second harmonic ultrasound system. Concordance between MCE and SPECT segmental scores was 92%, and agreement for the presence or absence of coronary artery disease was 86%. The conclusion from the investigators was that the assessment of myocardial perfusion with MCE is similar to that provided by SPECT imaging.[23]

ADVANCES IN OPTISON IMAGING

It has proved difficult with early generation ultrasound technology to provide perfusion images that can be easily interpreted by the echocardiographic community at large. Postprocessing algorithms such as background subtraction and color coding appear to improve the signal substantially[14,23] but are time consuming and not widely available. To address this, the ultrasound manufacturers are rapidly advancing this field with refined harmonic and broad-band technology (Figure 4). All of these exploit further the resonant properties of Optison, and clinical trials are underway to prove their clinical utility and gain regulatory approval for Optison as a myocardial perfusion agent.

Despite the excitement over the potential of perfusion imaging with Optison, it is important to remember the value of wall motion and perfusion. To that end, Porter et al[23] at University of Nebraska and others are combining wall motion and perfusion with Optison in a modality described as accelerated intermittent imaging. By imaging at a frame rate between 30 Hz (real time) and <1 Hz (intermittent) while stressing the patient and administering Optison, they are able to visualize new wall motion abnormalities simultaneously with perfusion abnormalities because this frame rate "destroys" fewer microspheres than real-time imaging (Figure 5). Other techniques including quantitation of myocardial blood volume by measuring videointensity at various dual-trigger intervals[24] may also prove useful in the clinical setting.

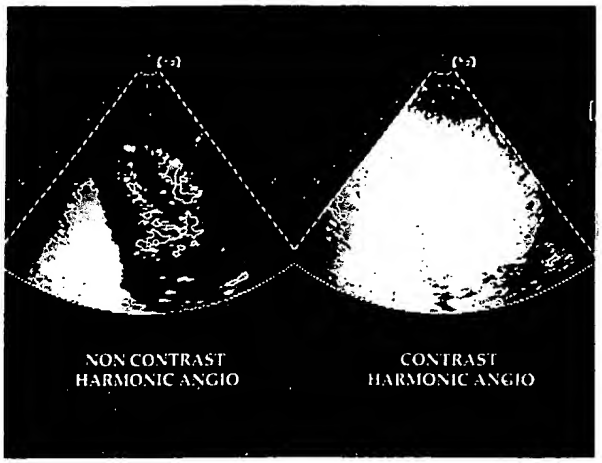

FIGURE 4. Doppler harmonic mode before (*left*) and after Optison (*right*). This modality measures the amplitude of signal produced by Optison's interaction with ultrasonic energy, producing diffuse signal throughout the normally perfused myocardium. ANGIO = angiograph.

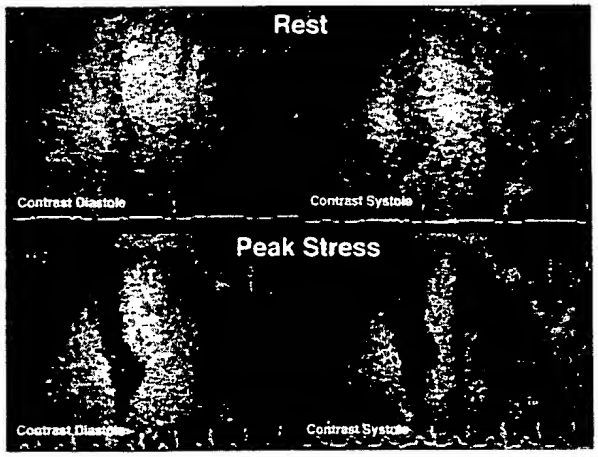

FIGURE 5. Rest and stress Optison images during accelerated intermittent imaging in a patient with an occluded graft to the left anterior descending coronary artery. Note the homogenous contrast uptake in the septal wall at rest in diastole and systole and the lack of contrast at peak stress, reflecting the stress-induced hypoperfusion. The perfusion abnormality, as expected, preceded the wall-motion abnormality.

SAFETY

Adverse events reported for Optison are not dissimilar to those noted for Albunex.

Adverse reactions: Optison was administered in clinical studies in 279 patients. In these patients, including normal subjects and those who received cumulative volumes up to 44 mL, 47 (16.8%) reported at least 1 adverse event. Of these, 1 adverse event was serious and required treatment with antihistamines for

hypersensitivity manifestations of dizziness, nausea, flushing, and temperature evaluation. Deaths were not reported during the clinical studies. Of the reported adverse reactions after the use of Optison, the most frequently reported were headache (5.4%), nausea and/or vomiting (4.3%), warm sensation or flushing (3.6%), and dizziness (2.5%).

Since its commercial release in January 1998, tens of thousands of injections of Optison have been administered with only a handful of unexpected adverse events reported to the manufacturer, none of which was severe. The safety profile, as demonstrated in the pivotal clinical trials is good, acceptable for an imaging agent, and similar to the previously approved agent, Albunex.

CONCLUSION

Optison is a safe and effective ultrasound contrast agent that reliably improves endocardial delineation in all imaging modalities. It has been shown to convert unreadable resting and stress studies to diagnostic quality. It further enhances the images obtained with second harmonic imaging, and use can result in cost savings by the avoidance of additional testing.

Optison shows great promise as a perfusion agent for the evaluation of patients with known or suspected ischemic heart disease. It is clear that the future of echocardiography, and its broader use in ischemic heart disease, will rely on imaging with ultrasound contrast agents such as Optison.

1. Cohen JL, Cherif J, Segar DS, Gillam LD, Gottdiener JS, Hausnerova E, Bruns DE. Improved left ventricular endocardial border delineation and opacification with Optison (FS069), a new echocardiographic contrast agent. *J Am Coll Cardiol* 1998;32:746–752.

2. Dolan MS, Puri S, Flanagan J, Vrain JA, Havens E, Labovitz AJ. Comparison of harmonic power, harmonic and fundamental imaging with echo contrast on left ventricular opacification [abstract]. *Echocardiography: J Cardiovasc Ultrasound Allied Tech* 1998;15(suppl 4):616.

3. Hausnerova E, Gottdiener JS, Kuvelis MT. Videodensitometric analysis of LV opacification with Optison vs Albunex influence of LV function, pulmonary disease, obesity and echogenicity [abstract]. *J Am Coll Cardiol* 1998;31(suppl A):440A.

4. Mulvagh SL, Rainbird AJ, Al-Mansour HA, Klarich KW, Foley DA, Kuvelas T, Miller JJ. Harmonic dobutamine stress echocardiography: does contrast make a difference in diagnostic feasibility and accuracy? *Circulation* 1998;98(suppl): I-356.

5. Reilly JP, Tunick PA, Timmermans RJ, Stein B, Rosenzweig BP, Kronzon I. Contrast echocardiography clarifies uninterpretable wall motion in intensive care unit patients. *J Am Coll Cardiol* 2000;35:485–490.

6. Ellahham S, Hausnerova E, Gottdiener J. Intravenous Optison (FS069) enhances pulmonary vein flow velocity signals: a multicenter study. *Clin Cardiol* 2000;23:91–95.

7. Dolan MS, Vrain JA, Puri S, Flanagan J, Havens E, Habermehl K, Labovitz AJ. Effect of Optison on endocardial visualization and accuracy of ejection fraction determination during stress echocardiography [abstract]. *Echocardiography: J Cardiovasc Ultrasound Allied Tech* 1998;15:675.

8. Rubin DN, Wharton WW, Segar DS, Cohen JL, Hecht HS, Kuvelas MT, West HE, Grayburn P. Conversion of uninterpretable exercise stress echo segments to diagnostic quality using Optison contrast microspheres [abstract] Proceedings of the 71st Scientific Sessions. *Circulation* 1998;98(suppl 17):I-83.

9. Shaw LJ, Ryan T, Cohen JL, Hecht HS, Grayburn PA. Estimation of myocardial perfusion with Optison contrast echocardiography [abstract]. *Circulation* 1998;98(suppl 17):214.

10. Hend A, Al-Mansour HA, Pumper GM, Foley DA, Kukuzke JA, Nelson JM, Mulvagh SL. Harmonic contrast echocardiography at peak dobutamine stress: endocardial visualization is improved by a 3-fold magnitude [abstract]. *J Am Soc Echocardiogr* 1998;11:544.

11. Mulvagh SL, Hend A, Al-Mansour HA, Pumper GM, Kukuzke JA, Stussy VL, Foley DA. Practical impact of improved endocardial border visualization during contrast dobutamine stress echocardiography [abstract]. *J Am Soc Echocardiogr* 1998;11:525.

12. Davarashvili JI, Buscombe JR, Smith WL, Coghlan GJ, Evans TR, Lipkin DP. Initial experience with myocardial contrast dobutamine stress echo: comparison with stress myocardial perfusion SPECT [abstract]. *Eur Heart J* 1998; 19(suppl):332.

13. Dolan M, Puri S, Tamirisia K, Koshy S, Vrain JS, Havens E, Flanagan J, Kern M, Bransford T, Labovitz A. The effect of Optison on sensitivity and specificity of dobutamine contrast echocardiography in technically difficult patients [abstract]. *J Am Coll Cardiol* 1999;33(suppl A):411A.

14. Cotter B, Kwan OL, DeMaria AN. Non invasive detection of myocardial perfusion by new echocontrast agents [abstract]. *Cardiovasc Imag* 1996;8:275.

15. Dolan M, Puri S, Tamirisia K, Koshy S, Vrain JS, Havens E, Flanagan J, Kern M, Bransford T, Labovitz A. Increased detection of viable myocardium using myocardial perfusion analysis with dobutamine stress echocardiography in patients with resting wall motion abnormalities [abstract]. *J Am Coll Cardiol* 1999;33(suppl A):481A–482A.

16. Mor-Avi V, Lang RM, Robinson KA, Korcarz C, Ng AF, Vignon P, Akselrod S, Shroff SG. Contrast echocardiographic quantification of regional myocardial perfusion: validation with an isolated rabbit heart model. *J Am Soc Echocardiogr* 1996;9:156–165.

17. Dolan MS, Puri S, Wittry MD, Vrain JS, Flanagan J, Labovitz AJ. Comparison of myocardial contrast echocardiography with Tc99m SPECT in assessment of segmental myocardial perfusion [abstract]. *Echocardiography: J Cardiovasc Ultrasound Allied Tech* 1998;15(suppl 4):34.

18. Macioch JE, Sandelski J, Ostoic TA, Johnson MS, In M, Thew ST, Liebson PR, Becher H, Holoviak M, Feinstein SB. Clinically reproducible myocardial perfusion studies in 82 consecutive patients: correlation to cardiac cath or nuclear imaging in 36 patients [abstract]. *Echocardiography: J Cardiovasc Ultrasound Allied Tech* 1998;15(suppl 3):31.

19. Main ML, Escobar JF, Hall SA, Killam AL, Grayburn PA. Detection of myocardial perfusion defects by contrast echocardiography in the setting of acute myocardial ischemia with residual antegrade flow. *J Am Soc Echocardiogr* 1998;11:228–235.

20. Meza M, Greener Y, Hunt R, Perry B, Revall S, Barbee W, Murgo JP, Cheirif J. Myocardial contrast echocardiography: reliable, safe, and efficacious myocardial perfusion assessment after intravenous injections of a new echocardiographic contrast agent. *Am Heart J* 1996;132:871–881.

21. Mobarek S, Kates M, Meza M, Moreno C, Revall S, Barbee W, Murgo JP, Cheirif J. Identification of perfusion abnormalities using FS069, a novel contrast agent, in conscious dogs. *Echocardiography: J Cardiovasc Ultrasound Allied Tech* 1997;14:337–344.

22. Kaul S, Senior R, Dittrich H, Raval U, Khattar R, Lahiri A. Detection of coronary artery disease with myocardial contrast echocardiography. *Circulation* 1997;96:785–792.

23. Porter T, Li S, Jiang L, Grayburn P, Deligonul U. Real-time visualization of myocardial perfusion and wall thickening in human beings with intravenous ultrasonographic contrast and accelerated intermittent harmonic imaging. *J Am Soc Echocardiogr* 1999;12:266–271.

24. Wei K, Jayaweera AR, Firoozan S, Linka A, Skyba DM, Kaul S. Quantification of myocardial blood flow with ultrasound-induced destruction of microbubbles administered as a constant venous infusion. *Circulation* 1998;10;97:473–483.

Heart 1999;81:636–641

Evaluation of myocardial, hepatic, and renal perfusion in a variety of clinical conditions using an intravenous ultrasound contrast agent (Optison) and second harmonic imaging

J Hancock, H Dittrich, D E Jewitt, M J Monaghan

Abstract

***Objective*—To assess the potential of intravenous Optison, a second generation ultrasound contrast agent, and various ultrasound imaging modes to determine myocardial, kidney, and liver perfusion in normal subjects and patients with left ventricular dysfunction or chronic pulmonary disease together with renal or hepatic dysfunction.**

***Methods*—Five normal subjects and 20 patients underwent grey scale echocardiographic imaging of myocardium, kidney, and liver during 505 intravenous injections of Optison. Images were assessed qualitatively by two independent observers and quantitatively using video densitometry to determine the peak contrast enhancement effect.**

***Results*—Qualitative analysis showed that intermittent harmonic imaging was superior to either conventional fundamental or continuous harmonic imaging for all organs. Quantitative analysis showed that the peak change in echocardiographic intensity *v* baseline during continuous harmonic imaging was 11 units for myocardium ($p < 0.03$), 7 units for kidney (NS), and 14 units for liver ($p < 0.05$). During intermittent harmonic imaging the peak change was significantly greater, being 33 units for myocardium ($p < 0.0001$), 24 units for kidney ($p < 0.0002$), and 16 units for liver ($p < 0.001$).**

***Conclusions*—Organ tissue perfusion can be demonstrated following intravenous injection of Optison, particularly when used in combination with intermittent harmonic imaging techniques. This contrast agent is effective in a variety of clinical conditions.**

(*Heart* 1999;81:636–641)

Keywords: ultrasound; contrast enhancement; echocardiography; Optison

Department of Cardiology, King's College Hospital, Denmark Hill, London SE5 9RS, UK
J Hancock
D E Jewitt
M J Monaghan

Molecular Biosystems Inc, 10030 Barnes Canyon Road, San Diego, California, USA
H Dittrich

Correspondence to:
Dr Hancock.

Accepted for publication
19 November 1998

To date, myocardial contrast echocardiography has had limited clinical applications because of the need to inject the contrast agent directly into the aorta or coronary arteries. More recently, the development of new ultrasound contrast agents with microsphere bubbles which are stable in vivo and which can retain their backscatter properties during transpulmonary transit has made endocardial definition and myocardial opacification following intravenous injection possible.[1]

In addition there have been advances in ultrasound technology. First, the discovery that, at diagnostic ultrasound intensities, contrast microbubbles resonate and create backscatter signals, not only at the fundamental (transmitted) frequency but at harmonics of that frequency.[2] Although the backscatter is less at the harmonic frequencies than at the fundamental frequency, because tissue produces a smaller harmonic signal than contrast microspheres, the difference between tissue alone and tissue plus contrast agent is enhanced. Thus the development of imaging software and ultrasound transducers which transmit at the fundamental frequency and receive at the second harmonic frequency (twice the fundamental frequency) has improved the detection of intravenously injected contrast agents within the myocardium.[3] Second, it has been observed that diagnostic ultrasound intensities may destroy contrast microspheres so that, by reducing the transmitting ultrasound intensity, the contrast effect is enhanced.[4] This can be achieved by imaging intermittently using only one ultrasound frame for every one to five cardiac cycles. This allows destroyed contrast agent to be replenished in the tissue between frames and enhances the contrast effect.

The combination of the new transpulmonary contrast agents and the new ultrasound imaging techniques may make it possible to evaluate organ perfusion in humans following intravenous injections.

The aim of this study was to assess the efficacy of the contrast agent Optison (Molecular Biosystems Inc, San Diego, USA) in human subjects for myocardial, liver, and kidney perfusion following intravenous injection. Several animal studies of this agent have been reported,[1 5 6] as well as initial safety studies in humans.[7 8] Studies examining the role of this agent in patients have focused mainly on myocardial perfusion in the setting of coronary artery disease.[6] Little is known about myocardial perfusion in patients with left ventricular dysfunction or chronic pulmonary disease, which may affect the transpulmonary passage of the contrast agent. The perfusion of other organs such as liver or kidney following intravenous injections of agents such as Optison has not been studied in detail in the human, particularly in the setting of hepatic or renal

Table 1 Left ventricular ejection fraction, pulmonary artery systolic pressure, and hepatic and renal function in the patients studied

Patient	EF (%)	PASP (mm Hg) + JVP	Bilirubin (μmol/l)	AST (IU/l)	ALP (IU/l)	Creatinine (μmol/l)
6	35–40					86
7	30–35					100
8	20–25					100
9	20–25					105
10	25–30					101
11	30–35					120
12	20–25					114
13	25–30			302		120
14	20–25					106
15	25–30			79	863	272
16	45–50	80	38.3		192	115
17	30–35	64				101
18	25–30	50				111
19	35–40	50		423	177	302
20	25–30	50				313
21	40–45	52				175
22	35–40	64				239
23	35–40					544
24	40–45	50				347
25	30–35		44.6	114	145	151

ALP, alkaline phosphatase (normal 30–120 IU/l); AST, aspartate aminotransferase (normal < 41 IU/l); EF, ejection fraction; PASP, pulmonary artery systolic pressure (mm Hg + jugular venous pressure (JVP)).

dysfunction, though there are a few reports of animal studies with such agents.[10-12] These agents also have the ability to delineate tumours within organs.[13] Second harmonic imaging enhances these images.[14] Optison can demonstrate liver and kidney perfusion with second harmonic imaging in animals, but its clinical efficacy has not been studied.[15] In this study we set out to address these points.

Methods

PATIENTS

Twenty five subjects were enrolled. Five were normal male volunteers (mean age 36 years, range 27 to 42). Twenty were patients (18 male, two female; mean age 58 years, range 32 to 78) with moderate to severe left ventricular dysfunction (ejection fraction < 40%) or chronic pulmonary disease (including pulmonary hypertension with pulmonary artery systolic pressure > 40 mm Hg) or both. Ten had normal biochemical profiles and 10 had impaired renal function, impaired liver function on biochemical testing, or both (table 1). The project was approved by King's Healthcare ethics committee. All subjects gave written informed consent. Inclusion criteria included age 18 to 80 years and adequate baseline ultrasound images of heart, liver, and kidney. Exclusion criteria included pregnancy, lactation, known hypersensitivity to blood, blood products, or albumin, unstable angina, and acute myocardial infarction.

All subjects had a physical examination, a 12 lead ECG, blood biochemical and haematological analysis, and urinalysis before the study and then at 30 minutes and 24 hours after the study.

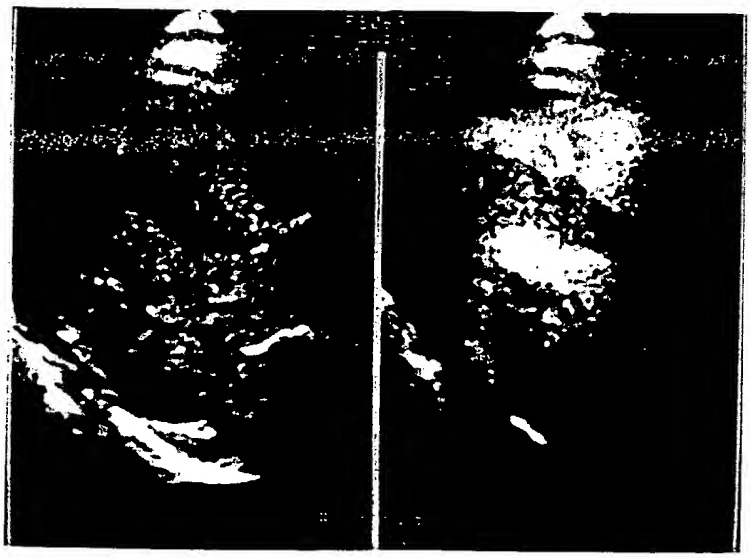

Figure 1 Myocardial opacification early (left) and late (right) after a 1 ml injection of Optison during continuous harmonic imaging. Parasternal long axis and apical four chamber views are shown. Early after the injection of Optison, right and left ventricular cavities are opacified but no myocardial opacification is seen. Late after the injection, good myocardial opacification is demonstrated (score of 3 on visual analysis).

CONTRAST AGENT

Optison is a second generation contrast microsphere agent made by Molecular Biosystems Inc (MBI). It consists of octafluoropropane filled microspheres of mean diameter 2–5 μm in a concentration of $1–10\times10^8$ particles/ml, prepared by the sonication of 1% human albumin in the presence of perfluoropropane (PFP). This agent has excellent backscatter properties and long in vivo persistence following intravenous injection. Clinical trials exemption was approved. Subsequently, Optison has been given a licence for sale in Europe and America.

The agent was kept suspended by continuous gentle agitation. Intravenous injections in a range of 0.1–5 ml (total 45 ml per patient) were given through an antecubital vein at a rate of less than 1 ml/s, followed by a saline flush. Any adverse events during the study and up to 24 hours afterwards were noted. After the first eight patients it became apparent that it was unnecessary to use 45 ml of contrast agent per patient in order to obtain satisfactory organ tissue enhancement, so the maximum dose was reduced to 20 ml per patient. The dose was adjusted to achieve maximum contrast enhancement effect with minimal attenuation (optimal perfusion volume). Attenuation occurs when the concentration of contrast agent within the right or left ventricular cavity is so great that it creates an acoustic shadow behind it, obscuring all tissues which are further away from the ultrasound transducer. This effect almost always occurs at doses sufficient to produce contrast enhancement within the tissue but its persistence can be reduced by reducing the dose of contrast agent, such that attenuation occurs in the early phase following intravenous injection during peak opacification of the right and left ventricular cavities, but has receded by the time of peak contrast opacification of the tissue.

Overall, 505 intravenous injections of Optison were given (mean 20 per subject).

ULTRASOUND IMAGING

A prototype Hewlett Packard Sonos 2500 system (Hewlett Packard Inc, Andover, Massachusetts, USA) and an ATL HDI 3000 (ATL Inc, Bothell, Washington, USA) were used for grey scale ultrasonic imaging of myocardium, liver, and kidney. Images were recorded just

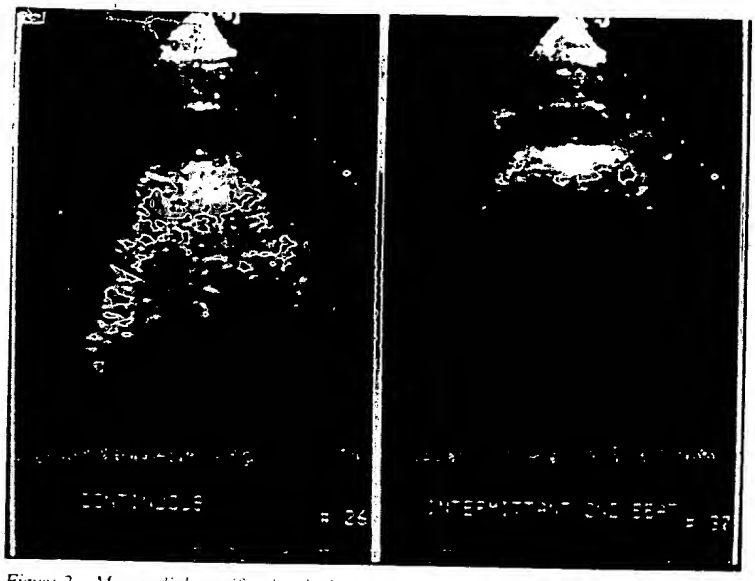

Figure 2 Myocardial opacification during continuous (left) and intermittent (right) harmonic imaging every second cardiac cycle following a 1ml injection of Optison. During continuous harmonic imaging, opacification of the left ventricular cavity, septum, and lateral wall is seen, but no opacification at the apex. During intermittent harmonic imaging, less contrast agent is destroyed, which results in visible perfusion at the apex and increased cavity attenuation.

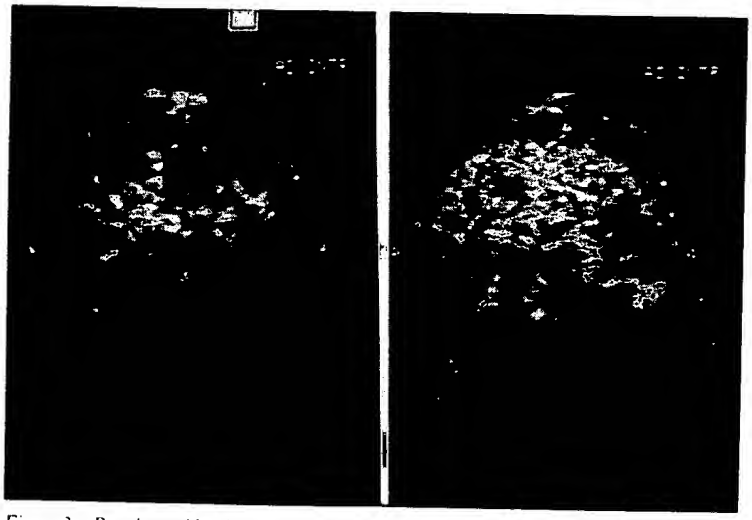

Figure 3 Renal opacification during continuous (left) and intermittent (right) harmonic imaging every second cardiac cycle following a 1 ml injection of Optison. During intermittent imaging, less destruction of contrast agent results in increased tissue opacification

before and during intravenous injection of Optison until the contrast effect had dissipated. Both conventional fundamental and second harmonic imaging modes were used. Ultrasound transmitted intensity was varied between 5% and 100% of maximum available power (mechanical index 0.2–1.6). Second harmonic imaging was achieved by using a transducer which transmits at 1.8 MHz and receives at 3.6 MHz for the Hewlett Packard system, and transmits at 1.67 MHz and receives at 3.3 MHz for the ATL machine. Continuous and intermittent harmonic imaging modes were used. Intermittent imaging was performed at end systole every one, two, or five cardiac cycles. In this way destroyed contrast microspheres are replenished in the tissues between imaging frames. Images were recorded on super VHS video tape (as well as on optical disk) for offline analysis.

DATA ANALYSIS

Data were analysed qualitatively by two independent observers using a visual contrast enhancement scoring system where 0 = no enhancement, 1 = faint enhancement, 2 = moderate enhancement, 3 = good enhancement, and 4 = attenuation, to determine the optimal perfusion volume for each organ in each subject. This was done in fundamental imaging mode. The peak contrast enhancement effect was scored using the same scale, once any effect of attenuation had subsided (that is, a score of 4 was not considered in assessing the peak contrast enhancement effect). This was done for all organs in all subjects at the optimal perfusion volume and using all imaging modes. Any disagreement between observers was resolved by consensus.

Video densitometry was performed using a Pentium PC workstation and Osiris medical imaging software, version 3.0, developed by the University Hospital of Geneva. This is available as shareware at internet site http://expasy.hcuge.ch/UIN. A still video frame was "grabbed" and the ultrasonic grey scale level, representing echocardiographic intensity, measured within a region of interest drawn in a segment of the relevant tissue both before contrast injection and at peak contrast effect. This was repeated for all organs and all harmonic imaging modes for each subject. Organ perfusion was assessed by the change from baseline grey scale intensity.

STATISTICAL ANALYSIS

The pre and peak contrast effects for each organ in each imaging mode were compared using a paired t test. Comparisons between imaging modes were performed using an unpaired t test. A probability (p) value of < 0.05 was taken to imply a significant difference.

Results

QUALITATIVE ANALYSIS

Visual analysis of the videotaped images was used to assess the optimal perfusion volume, deriving maximum opacification with minimal attenuation for each organ. This was 0.1–0.5 ml for myocardium, 1 ml for kidney, and 1–2 ml for liver in all patient groups.

With two observers using a visual scoring system to evaluate organ perfusion (fig 1), fundamental and continuous second harmonic imaging of all organs elicited faint opacification with contrast, with a score of 1.07 and 1.32, respectively. In contrast, intermittent harmonic imaging provided the best visual evaluation of perfusion for all organs (figs 2 and 3), achieving a score of 2.23 ($p < 0.001$) representing moderate to good (fig 4), though there was no significant difference between the intermittent imaging modes. For example the scores for continuous harmonic and intermittent harmonic imaging every one, two, or five beats for myocardium were 1.32, 2.69, 2.91, and 3.33, respectively ($p < 0.0001$ for all intermittent modes v continuous imaging).

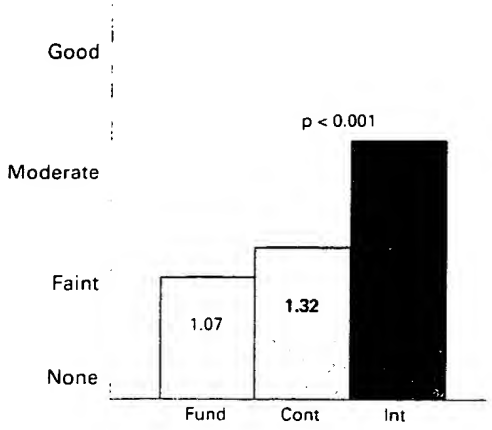

Figure 4 Qualitative analysis of peak contrast enhancement effect for all organs at the optimal perfusion volume using a visual scoring system. 0 = none; 1 = faint; 2 = moderate; 3 = good. Cont, continuous harmonic imaging; Fund, fundamental imaging; Int, intermittent harmonic imaging.

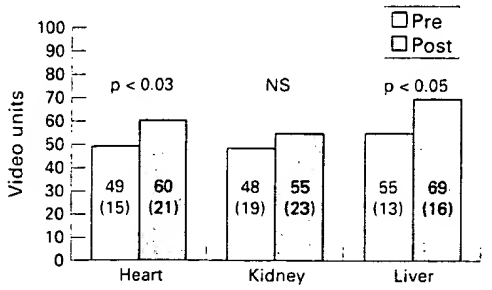

Figure 5 Peak video grey scale intensity at baseline and after contrast injection at the optimal perfusion volume for all organs for continuous harmonic imaging. Values are mean (SD).

INTEROBSERVER VARIABILITY

The two independent observers achieved agreement in 162 of 225 scores (72%) when performing qualitative assessment of the contrast enhancement effect at the optimal perfusion volume. Agreement to within one score was achieved in 196 of 225 assessments (87%). This represents good to excellent agreement.

QUANTITATIVE ANALYSIS

The results of the videodensitometric analysis to assess the change in peak harmonic grey scale from baseline following contrast injection for continuous harmonic imaging for all organs are shown in fig 5. The mean change in peak harmonic grey scale was 11 video grey scale units (95% confidence interval 2.4 to 19) for heart ($p < 0.03$), 7 units (−8 to 22) for kidney (NS), and 14 units (1.7 to 27) for liver ($p < 0.05$).

The results for intermittent harmonic imaging (every second cardiac cycle) for all organs are shown in fig 6. The change in peak harmonic grey scale level was 33 video grey scale units (95% confidence interval 25 to 41.5) for heart ($p < 0.0001$), 24 units (12 to 37) for kidney ($p < 0.0002$), and 16 units (6.5 to 26.5) for liver ($p < 0.001$), showing a significant improvement in contrast effect comparing all intermittent modes with continuous imaging.

Comparing the contrast enhancement effect in each organ between the different patient

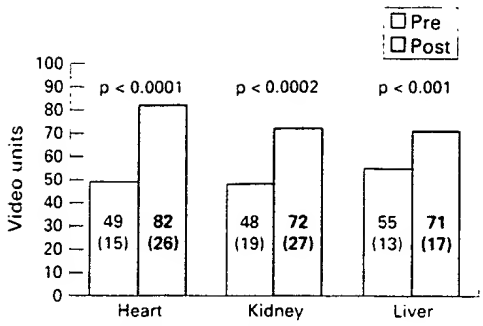

Figure 6 Peak video grey scale levels at baseline and following contrast injection at the optimal perfusion volume for all organs during intermittent harmonic imaging every second cardiac cycle. Values are mean (SD).

subgroups did not reveal any significant differences. The peak change in contrast enhancement effect in the heart was 14.6 for normal subjects, 16.9 for those with impaired left ventricular function and/or chronic pulmonary disease and normal hepatic and renal function, and 20.6 for those with left ventricular dysfunction, chronic pulmonary disease, hepatic impairment, and/or renal impairment (NS). For the kidney these figures were 14.2, 16, and 24.9 (NS), and for the liver they were 13, 17.2, and 23.6, respectively (NS).

ADVERSE EVENTS

One subject who was diabetic with very poor left ventricular function had a rigor two hours after the study and developed a fever of 38.8°C and flu-like symptoms six hours after the study. Paracetamol was given to lower the temperature. All symptoms had resolved by the following day. There were no changes on the 12 lead ECG or biochemical profile. However the white cell count rose from 8.87×10^9/litre before the study to 17.06×10^9/litre 30 minutes after the study, with a neutrophilia of 91.7%. Blood cultures were negative. This picture had resolved 24 hours after the study and may have reflected an adverse reaction to the contrast agent.

No other subjects had adverse reactions and there were no changes in their physical examination, 12 lead ECG, or biochemical and haematological profiles.

Discussion

The detection of myocardial (or other organ tissue) perfusion following intravenous contrast injection has previously been limited by the ability of the contrast agent to survive transpulmonary passage, as well as by the limitations of the ultrasound technology to detect the presence of contrast within the tissue. More recently, second generation contrast agents have been developed which can survive transpulmonary transit. Optison is such an agent, consisting of albumin microspheres filled with octafluoropropane. This is a high molecular weight gas which does not diffuse out of microbubbles as readily as air. This means that the microbubbles retain their shape and backscatter properties during transpulmonary transit, unlike first generation ultrasound contrast agents that contain air, which is highly

diffusible and rapidly escapes from bubbles when mixed with blood; since the backscatter of a bubble is related exponentially to its radius, loss of air results in decreased bubble size which, in turn, leads to a decrease in its backscatter properties. Microbubble destruction following resonance would cause further loss of backscatter in a less stable contrast agent.

Contrast microbubbles resonate at transmitted ultrasound frequencies. They release ultrasound energy both at this frequency and at harmonics of this frequency. Unlike tissue, therefore, they have non-linear ultrasound backscatter properties. The development of ultrasound transducers that can transmit at one frequency and receive at the second harmonic of (that is, twice) that frequency has enhanced the ability to detect contrast microbubbles within the tissue. This is because, although the signal amplitude or backscattered energy is greater at the fundamental (transmitted) frequency, the difference between the tissue effect and the contrast effect is much greater at the second harmonic frequency since tissue generates less second harmonic signal than contrast.[16]

Many contrast microbubbles are destroyed by diagnostic ultrasound intensities. Therefore, reducing ultrasound intensity—either by reducing the transmit power or by reducing the time between insonations—allows accumulation of contrast agent and enhances the ability to detect its presence within the organ tissue of interest.[4 6 17]

Myocardial opacification following intravenously injected Optison has been studied with these ultrasound techniques in animal models. Studies in dogs during coronary occlusion have shown perfusion defects during occlusion which resolve following restoration of normal flow.[8] Perfusion defects also correlate well with wall motion abnormalities and risk area determined by technetium scanning during coronary occlusion.[18] A study comparing Optison with its parent compound Albunex showed that Optison was superior for evaluation of left ventricular chamber opacification and endocardial border delineation.[19] Intermittent harmonic imaging during intravenous Optison injection in dogs shows good myocardial opacification.[6] Perfusion defects occurred during adenosine induced hyperaemia in the presence of coronary stenoses during intermittent harmonic imaging which were difficult to measure during fundamental or continuous harmonic imaging.[5] The magnitude of the perfusion defect using intermittent harmonic imaging correlated well with the magnitude of flow mismatch when radiolabelled microspheres were used, as well as with risk area and infarct size following coronary occlusion and reperfusion determined at necropsy. One study in humans has shown that myocardial contrast echocardiography following intravenous Optison can define the presence of coronary artery disease at rest and during pharmacological stress. The location of perfusion defects and their clinical relevance was comparable to the results of technetium sestamibi single photon emission computed tomography (SPECT).[9]

In the present study we have not examined the ability of Optison to demonstrate coronary artery disease, but rather its use in demonstrating myocardial, kidney, and liver perfusion at low doses in a variety of clinical conditions which may affect the transpulmonary passage, uptake, and persistence of the contrast agent in these organs. Our study has shown that Optison can opacify organ tissue at very low doses (as little as 0.1 ml) following intravenous injection. These doses are much lower than are necessary with the parent compound, Albinex. Previous studies with Optison in open chest dogs with the ultrasound transducer on the epicardial surface have used similar doses. The only other published study in human myocardium used doses of 0.5 or 1 ml. The optimal perfusion volume for each organ was less than 2 ml during fundamental imaging. Intermittent second harmonic imaging, insonating at one frame every second cardiac cycle, provided the optimum imaging mode for all organs, increasing the peak change in video grey scale intensity from 11 to 33 units for myocardium ($p < 0.0001$), from 7 to 24 units for kidney ($p < 0.0002$), and from 14 to 17 units for liver ($p < 0.002$) compared with continuous harmonic imaging. This was also appreciated visually by two independent observers, intermittent harmonic imaging achieving a score of 2.33 (moderate to good) compared with fundamental and continuous second harmonic imaging, which achieved scores of 1.07 and 1.32 respectively (faint). The presence of left ventricular function or chronic pulmonary disease with pulmonary hypertension did not seem to reduce the ability of the contrast agent to demonstrate organ perfusion, the peak change in video grey scale being similar to that in normal subjects (14.6 *v* 16.9 for myocardium (NS), 14.2 *v* 16 for kidney (NS), and 13 *v* 17.2 for liver (NS)). There were no significant differences in kidney and liver enhancement compared with normal subjects in the presence of renal or hepatic dysfunction (14.2 *v* 24.9 for kidney and 13 *v* 23.6 for liver). Rather, any difficulty in perceiving contrast enhancement seemed to depend entirely on the imaging mode (intermittent harmonic imaging being superior in all cases) and the quality of the baseline image.

LIMITATIONS OF THE STUDY

While this study involved only 25 patients, 505 intravenous injections of Optison were given, which did enable an analysis of the optimum perfusion volume for each organ as well as a comparison of the tissue contrast enhancement between the different imaging modes. Since harmonic imaging was so superior to fundamental imaging, an analysis of intermittent fundamental imaging was not performed. Although we made some comparisons between the patient subgroups with respect to the contrast enhancement effect (five normal subjects *v* 10 subjects with impaired left ventricular function and/or chronic pulmonary disease and normal renal and hepatic function, and 10

subjects with abnormal renal and/or hepatic function), these groups are rather small. Certainly, more detailed statistical analysis of subgroups has not been possible.

FUTURE DIRECTIONS

Since intravenous Optison can demonstrate organ perfusion in patients with a variety of clinical conditions, particularly in conjunction with intermittent harmonic imaging, it may have many uses in the clinical setting. As has been shown, it may be useful in evaluating reversible ischaemia in patients with ischaemic heart disease. It may also be useful after myocardial infarction to determine non-invasively which patients have viable myocardium in the territory of their infarct related artery and so might benefit from invasive investigation. It should be possible to determine the patency of the infarct related artery, which would rationalise invasive management.

CONCLUSIONS

In this study we have shown the ability of an ultrasound contrast agent such as Optison to opacify myocardium, kidney, and liver tissue following low dose intravenous injection. This is possible even in patients with conditions which may affect the transpulmonary transit of the agent, the circulation of the agent to the tissues, and its ability to opacify the tissues. Intermittent second harmonic imaging enhances this effect. The increased availability of this imaging technology will enable second generation contrast agents such as Optison to become a useful non-invasive tool to evaluate organ perfusion in a variety of clinical conditions. This may be more cost effective and acceptable to patients than techniques currently available.

HD is a full time employee of Molecular Biosystems Inc.

1 Skyba DM, Camarano G, Goodman NC, *et al.* Hemodynamic characteristics, myocardial kinetics and microvascular rheology of FS069, a second generation echocardiographic contrast agent capable of producing myocardial opacification from a venous injection. *J Am Coll Cardiol* 1996;28:1292–300.

2 Mulvagh SL, Foley DA, Aeschbacher BC, *et al.* Second harmonic imaging of an intravenously administered echocardiographic contrast agent. Visualisation of coronary arteries and measurement of coronary blood flow. *J Am Coll Cardiol* 1996;27:1519–25.

3 Kaul S. New developments in ultrasound systems for contrast echocardiography. *Clin Cardiol* 1997;20(suppl 1):I27–30.

4 Villarraga HR, Foley DA, Aeschbacher BC, *et al.* Destruction of contrast microbubbles during ultrasound imaging at conventional power output. *J Am Soc Echocardiogr* 1997;10:783–91.

5 Firschke C, Lindner JR, Wei K, *et al.* Myocardial perfusion imaging in the setting of coronary artery stenosis and acute myocardial infarction using venous injection of a second generation echocardiographic contrast agent. *Circulation* 1997;96:959–67.

6 Colon PJ, Richards DR, Moreno CA, *et al.* Benefits of reducing the cardiac cycle triggering frequency of ultrasound imaging to increase myocardial opacification with FS069 during fundamental and second harmonic imaging. *J Am Soc Echocardiogr* 1997;10:602–7.

7 Dittrich HC, Kuvelas T, Dadd K, *et al.* Safety and efficacy of the ultrasound contrast agent FS069 in normal humans: results of a phase I trial [abstract]. *Circulation* 1995; 92(suppl):I–464.

8 Meza MF, Greener Y, Aristizabal D, *et al.* Myocardial contrast echocardiography: safety of FS069, a new transpulmonary echocardiographic contrast agent. *J Am Soc Echocardiogr* 1994;7(suppl).

9 Kaul S, Senior R, Dittrich H, *et al.* Detection of coronary artery disease with myocardial contrast echocardiography. Comparison with ^{99m}Tc-sestamibi single photon emission computed tomography. *Circulation* 1996;96:785–92.

10 Leen E, McArdle CS. Ultrasound contrast agents in liver imaging. *Clin Radiol* 1996;51(suppl 1):35–9.

11 Jakobsen J. Echo enhancing agents in the renal tract. *Clin Radiol* 1996;51(suppl 1):40–3.

12 Albrecht T, Cosgrove DO, Correas JM, *et al.* Renal, hepatic and cardiac enhancement on Doppler and gray scale sonograms obtained with EchoGen. *Acad Radiol* 1996;3(suppl 2):S198–200.

13 Kono Y, Moriyasu F, Nada T, *et al.* Gray scale second harmonic imaging of the liver: a preliminary animal study. *Ultrasound Med Biol* 1997;23:719–26.

14 Cosgrove D. Ultrasound enhancement of tumours. *Clin Radiol* 1996;51:44–9.

15 Forsberg F, Goldberg BB, Liu JB, *et al.* On the feasibility of real time, in vivo harmonic imaging with proteinaceous microspheres. *J Ultrasound Med* 1996;15:853–60.

16 Burns PN. Harmonic imaging with ultrasound contrast agents. *Clin Radiol* 1996;51:50–5.

17 Wei K, Skyba DM, Firschke C, *et al.* Interactions between microbubbles and ultrasound: in vitro and in vivo observations. *J Am Coll Cardiol* 1997;29:1081–8.

18 Camarano GP, Ismail S, Goodman C, *et al.* Assessment of risk area during coronary occlusion and infarct size after reperfusion can be determined with myocardial contrast echocardiography using intravenous injections of FS069, a new contrast agent. *Circulation* 1994;90(suppl):I-68.

19 Dittrich HC, Bales GL, Killam AL. Comparative study for the left ventricular opacification and endocardial border delineation following intravenous administration of Albunex and FS069 in the anaesthetised canine. (Summary report 1041.) San Diego: Molecular Biosystems Inc, 1994.